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Mass Spectrometry in Structural and Stereochemical Problems. XXIV.[1] A Study of the Hydrogen Transfer Reactions Accompanying Fragmentation Processes of 11-Keto Steroids. Synthesis of Deuterated Androstan-11-ones[2]

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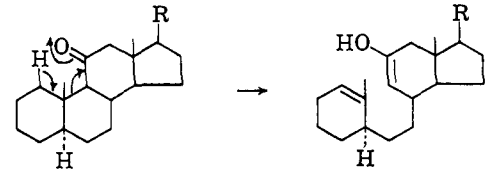
Fifteen mono- or poly-deuterated analogs of 5α-androstan-11-one have been synthesized with the label occurring in positions 1, 2, 3, 4, 5, 6, 8, 9, 12 and 17 and their mass spectra determined. As a result, it has been possible to assign plausible representations to the principal mass spectral fragments and to examine the occurrence of hydrogen transfer reactions. These have been found to be remarkably diverse and complicated, mechanisms being postulated for many of the fragmentation-*cum*-transfer processes. In the course of the synthetic work, it was possible to study in detail the enolization of 11-keto steroids, the ease of hydrogen abstraction proceeding in the order $9\alpha > 12\alpha \gg 12\beta$.

Introduction

In the first article of this series,[3] there were recorded the mass spectra of steroids with keto groups in all possible nuclear positions. By using substances with a keto function in a given location but with different substituents in other portions of the molecule, "labels" were available which permitted in many instances the assignment of plausible representations to the most characteristic mass spectral peaks. A similar approach was employed by us in examining the fragmentation behavior of steroidal sapogenins,[4] pentacyclic triterpenoids,[5] Δ^1- and Δ^4-3-keto steroids[1] and steroidal estrogens.[6] In the latter two instances, a few deuterated analogs were also prepared to secure some information on the hydrogen rearrangements, which often accompany fragmentation of organic molecules induced by electron bombardment.

For structural purposes, an over-all view of the mass spectral fragmentation process is often sufficient and the necessary information can be obtained usually by the type of "substituent labeling" alluded to above. However, it was emphasized already in our first paper[3] that proper mechanistic conclusions would require extensive labeling with deuterium. At this relatively early stage of the organic chemist's use of mass spectrometry,[7-11] it is very important to gather as much information as possible about the mechanism of such fragmentation processes—especially because of their resemblance to other high-energy reactions such as those found in photochemistry—and a knowledge of hydrogen rearrangements offers an important key to this problem. One of our aims[3] has been a very thorough study of the mass spectral behavior of steroids, commencing with monofunctional derivatives. For this purpose parallel investigations have been initiated with bicyclic model compounds[12,13] as well as with monoketonic steroids, which are labeled with deuterium in as many different locations as possible.

The present article is concerned with a monoketonic 5α-androstane, in which the fragmentation process is triggered, as well as largely controlled, by the presence of an 11-keto function. The initial observations[3] were performed with 5α-androstan-11-one (Ia) and 5α-pregnan-11-one (Ib), the different C-17 substituent serving as the guide to decide on the course of the principal fragmentation processes. These were shown to involve especially fission of the 9–10 bond and it was suggested,[3,14] therefore, that transfer of the C-1 hydrogen atom through a six-membered transition state (see arrows in I) represented the key step, subsequent fission being directed by the two newly formed double bonds.



Ia, R=H
b, R=C_2H_5

Recently[15] such a mechanism was also employed to rationalize the formation of the principal peak in the mass spectrum of cortisone and in fact was even used to explain the earlier noted[3] intensity differences in the mass spectra of 11-keto steroids epimeric at C-5. Such a mechanism (see I) is plausible and attractive. Nevertheless, it is absolutely indispensable that its operation be established by deuterium labeling at C-1, especially since completely analogous mechanisms can also be entertained[3,14] for 1-, 7- and 15-keto steroids. A synthetic program was, therefore, initiated to prepare C-1 deuterated 5α-androstan-11-one and, as shown below, its mass spectrum demonstrated the *virtual absence of any hydrogen transfer from C-1* in the genesis of the base peak (*m/e* 164) and relatively minor transfer in some of the less abundant peaks. It was then necessary to introduce labels into many other positions of the molecule and the resulting, rather formidable synthetic program is discussed first, before proceeding with a coverage of the mass spectral results and the consequent mechanistic deductions.

Synthesis of Deuterated 5α-Androstan-11-ones

The earlier syntheses[16] of 5α-androstan-11-one (Ia = IX) were not suitable for our purposes, since it was de-

(1) Paper XXIII, R. H. Shapiro, J. M. Wilson and C. Djerassi, *Steroids*, **1**, 1 (1963).

(2) We are indebted to the National Institutes of Health of the U. S. Public Health Service for financial support (Grants No. CRTY-5061 and A-4257).

(3) H. Budzikiewicz and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1430 (1962).

(4) H. Budzikiewicz, J. M. Wilson and C. Djerassi, *Monatsh.*, **93**, 1033 (1962).

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(13) E. Lund, H. Budzikiewicz, J. M. Wilson and C. Djerassi, *ibid.*, **85**, 1528 (1963).

(14) C. Djerassi, H. Budzikiewicz and J. M. Wilson, "Recent Applications of Mass Spectrometry in Steroid Chemistry," Proceed. Internat. Congress Hormonal Steroids, Milano, May, 1962, Academic Press, Inc., New York, N. Y., in press.

(15) Reference 10, pp. 343 and 347.

(16) N. Steiger and T. Reichstein, *Helv. Chim. Acta*, **20**, 817 (1937); F. Sondheimer, E. Batres and G. Rosenkranz, *J. Org. Chem.*, **22**, 1090 (1957).

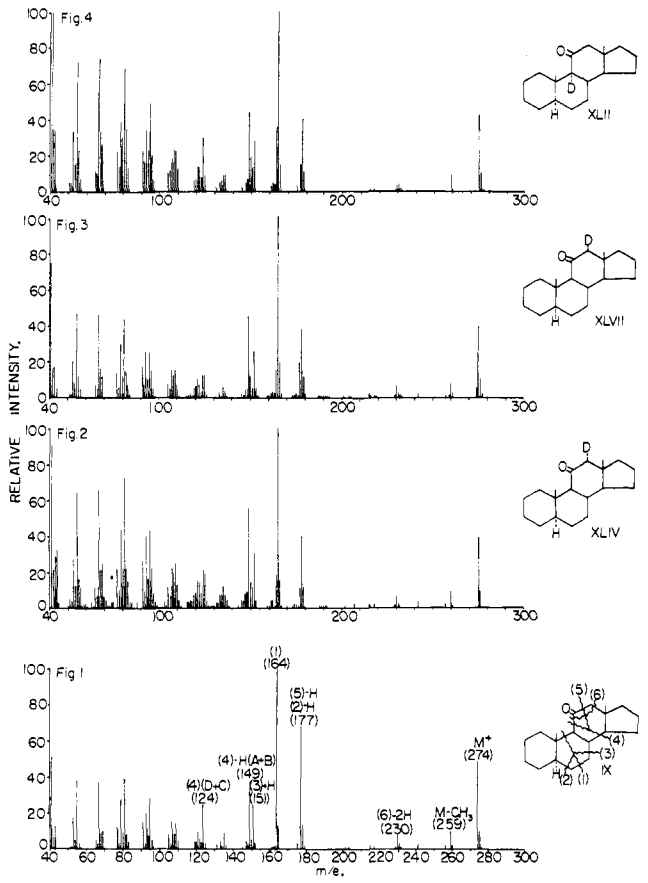

Fig. 1.—Mass spectrum of 5α-androstan-11-one (IX).
Fig. 2.—Mass spectrum of 12α-d_1-5α-androstan-11-one (XLIV), plotted so as to subtract the presence of non-deuterated IX.
Fig. 3.—Mass spectrum of 12β-d_1-5α-androstan-11-one (XLVII).
Fig. 4.—Mass spectrum of 9α-d_1-5α-androstan-11-one (XLII), plotted so as to subtract the presence of non-deuterated IX.

sirable to prepare the substance by a stepwise degradation which could be used to introduce deuterium at various nuclear positions. Since Beckmann rearrangement of Δ^{16}-20-keto steroid oximes provides an excellent route[17] to the corresponding 17-ketones, Δ^{16}-5α-pregnene-11,20-dione-3β-ol acetate (II)[18] was selected as the starting material[19] and transformed in good yield without isolation of intermediates to 5α-androstane-11,17-dione-3β-ol acetate (III). Treatment with ethanedithiol and boron trifluoride etherate[20] gave in nearly quantitative yield the 17-ethylene thioketal IV, which was desulfurized with freshly prepared W-7 Raney nickel catalyst[21] and directly saponified to furnish in 78% yield 5α-androstan-3β-ol-11-one (V). Oxidation at C-3 to the diketone VII and mercaptal formation (VIII) of the newly formed C-3 carbonyl group proceeded in excellent yield and desulfurization with a large excess of freshly prepared W-7 Raney nickel catalyst[21] yielded the required 5α-androstan-11-one (IX). However, when a lower catalyst/substrate ratio was employed, a slightly more polar substance was also formed, which by mass spectrometry (see Fig. 8) was shown to be Δ^2-5α-androsten-11-one (X),[22,22a] its structure being confirmed by independent synthesis through alumina treatment[23] of 5α-androstan-3β-ol-11-one tosylate (VI).

As noted above, confirmation of the originally postulated[3,14] fragmentation mechanism (I) required introduction of deuterium at C-1. Theoretically, 1,1-d_2-androstan-11-one might be available through desulfurization with deuterio-Raney nickel of the 1,1-ethylene thioketal of androstane-1,11-dione. For this purpose, an improved procedure was developed[24] for the conversion of 3-keto steroids to their 1-keto isomers so that the previously unknown androstane-1,11-dione could be prepared. The synthesis of the latter diketone, however, was not pursued when it was learned from Dr. R. Villotti (Syntex, S.A., Mexico City) that the hydrazine reduction[24,25] of a related 1α,2α-oxido-3,-11-diketone did not proceed in the desired direction. The monodeuterated 1-d_1-androstan-11-one (XV), however, proved to be readily available by the following route.

Bromination of 5α-androstane-3,11-dione (VII) in acetic acid led to the 2α-bromo derivative XVI which furnished Δ^1-5α-androstene-3,11-dione (XI) upon dehydrobromination with calcium carbonate[26] in dimethylacetamide solution. Catalytic deuteration of the unsaturated ketone XI in cyclohexane solution using palladium-on-charcoal catalyst provided 1,2-d_2-5α-androstane-3,11-dione (XII), the C-2 deuterium atom being exchanged readily (XIII) upon treatment with aqueous methanolic sodium hydroxide solution. The now superfluous C-3 oxygen function was removed in the usual manner by desulfurization of the mercaptal XIV to yield 1-d_1-5α-androstan-11-one (XV) of 80% isotopic purity, the mass spectrum of which demonstrated that no important deuterium transfer had occurred in the previously postulated[3,14,15] manner (arrows in I). Since it was not established whether the deuterium atom at C-1 in XV was axial or equatorial, or a mixture of both,[27,27a] this mass spectrometric experiment becomes mechanistically inconclusive in view of the possibility of stereospecific transfer of a 1β-deuterium atom. The 1-d_1-ketone XV does, however, contain a very useful label in ring A and its value in interpreting the mass spectrum (Fig. 1) of 5α-androstan-11-one will be discussed later.

The following method was next developed to make a 1,1-d_2-5α-androstan-11-one available. Reduction of the 2α-bromodione XVI with sodium borohydride[28]

(17) See (a) G. Rosenkranz, O. Mancera, F. Sondheimer and C. Djerassi, *J. Org. Chem.*, **21**, 520 (1956); (b) E. S. Rothman and M. W. Wall, *ibid.*, **25**, 1396 (1960).

(18) E. M. Chamberlin, W. V. Ruyle, A. E. Erickson, J. M. Chemerda, L. M. Aliminosa, R. L. Erickson, G. E. Sita and M. Tishler, *J. Am. Chem. Soc.*, **73**, 2396 (1951); C. Djerassi, E. Batres, J. Romo and G. Rosenkranz, *ibid.*, **74**, 3634 (1952).

(19) We are greatly indebted to Dr. B. A. Hems, Glaxo, Ltd. (Greenford, Middlesex) for a very generous gift of this substance.

(20) L. F. Fieser, *J. Am. Chem. Soc.*, **76**, 1945 (1954).

(21) H. R. Billica and H. Adkins, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 176.

(22) Olefin production in the desulfurization of mercaptals is very rare, the isolation (P. A. Plattner, A. Fürst and H. Els, *Helv. Chim. Acta*, **37**, 1399 (1954)) of Δ^1- and Δ^2-cholestene from desulfurization of 1,3,3-tribenzylmercaptocholestane representing the only relevant precedent. A detailed study of the various factors (age, quantity and method of preparation of catalyst) entering in this reaction is recorded elsewhere: C. Djerassi and D. H. Williams, *J. Chem. Soc.*, in press.

(22a) NOTE ADDED IN PROOF.—Other examples of olefin production in the desulfurization of mercaptals have recently been reported. See J. Fishman, M. Torigoe and H. Guzik, *J. Org. Chem.*, **28**, 1443 ((1963), and J. A. Steele, L. A. Cohen and E. Mosettig, *J. Am. Chem. Soc.*, **85**, 1134 (1963).

(23) G. H. Douglas, P. S. Ellington, G. D. Meakins and R. Swindells, *J. Chem. Soc.*, 1720 (1959).

(24) C. Djerassi, D. H. Williams and B. Berkoz, *J. Org. Chem.*, **27**, 2205 (1962).

(25) P. S. Wharton and D. H. Bohlen, *ibid.*, **26**, 3615 (1961).

(26) G. F. H. Green and A. G. Long, *J. Chem. Soc.*, 2532 (1961).

(27) The catalytic deuteration of ordinary Δ^1-3-keto-5α-steroids proceeds from the α-face (F. J. Schmitz and W. S. Johnson, *Tetrahedron Letters*, 647 (1962); H. J. Ringold, M. Gut, M. Hayano and A. Turner, *ibid.*, 835 (1962)), but since even rather slight structural changes can affect drastically this stereochemical picture (H. J. Brodie, M. Hayano and M. Gut, *J. Am. Chem. Soc.*, **84**, 3766 (1962)) it cannot be predicted *a priori* what effect the 11-keto function would have on the stereochemical course of the catalytic deuteration of XI.

(27a) NOTE ADDED IN PROOF.—The 1α-stereochemistry of the deuterium atom has now been established by n.m.r. spectrometry; see D. H. Williams, N. S. Bhacca and C. Djerassi, *J. Am. Chem. Soc.*, in press.

(28) Pertinent discussion of the course of the sodium borohydride reduc-

in ethanol furnished a mixture of the bromohydrin XVII and its 3α-epimer XVIII. Thin-layer chromatography showed only traces of more polar products, indicating that reduction of the 11-keto function under the conditions used was slight. The desired 2α-bromo-3β-alcohol XVII could be separated by alumina chromatography and, on treatment with potassium hydroxide in isopropyl alcohol solution, gave the 2β,3β-oxide XIX. Reduction of the oxide XIX with lithium aluminum hydride led to 5α-androstane-2β,11β-diol (XX), which was oxidized to the 2,11-dione XXI. It was proposed to exchange all of the enolizable hydrogens for deuterium in the 2,11-dione XXI, followed by preferential thioketal formation of C-2 and desulfurization to give an androstan-11-one fully deuterated at C-1.

On refluxing a 1% solution of 5α-androstane-2,11-dione (XXI) in deuteriomethanol containing 30% of deuterium oxide and 2% of dissolved sodium for two hours, a product was obtained containing 26% d_5-, 54% d_6- and 13% d_7-species. On increasing the reflux time to twenty-eight hours, the mass spectrum showed the presence of 13% d_5-, 38% d_6- and 49% d_7-components. It can be seen from these experiments that with a shorter reflux time predominantly a hexadeuterio product is obtained; prolonged treatment with base gives a heptadeuterio derivative, indicating that one hydrogen atom in the 2,11-dione is only replaced with difficulty. Later experiments, discussed below, in which the enolizable hydrogen atoms of 5α-androstan-11-one (IX) were replaced by deuterium in a similar base-catalyzed equilibration show that one of the hydrogen atoms at C-12 can be substituted only with difficulty.

The 1,1,3,3,9,12,12-d_7-5α-androstane-2,11-dione (XXII) was converted into the 2-monoethylene thioketal XXIII. Desulfurization with Raney nickel catalyst and exhaustive base treatment, to back-exchange deuterium from C-9 and C-12, provided 1,1,3,3-d_4-5α-androstan-11-one (XXIV), which was 60% pure with respect to the d_4-species XXIV, the remaining material being 10% d_2, 27% d_3 and 3% d_5.

In order to establish with certainty whether any transfer of deuterium was occurring from C-1 to the oxygen-containing portion of the molecule responsible for the base peak, it was now necessary to have available 3,3-d_2-5α-androstan-11-one (XXV), so that its mass spectrum could be compared with that of the 1,1,-3,3-d_4-analog XXIV. A feasible route to this substance appeared to be the desulfurization of 5α-androstane-3,11-dione 3-monoethylene thioketal (VIII) using Raney nickel containing active deuterium instead of hydrogen. A Raney nickel catalyst (approximating W-7 catalyst[21] in activity) was accordingly prepared utilizing deuterium oxide in place of water and washing the catalyst finally with deuteriomethanol instead of ethanol. Desulfurization of VIII using this catalyst, followed by treatment of the product with aqueous methanolic base to remove any deuterium incorporated in enolizable positions (due to alkali on the catalyst), did in fact afford 3,3-d_2-androstan-11-one (XXV) as the main isotopic species (48%), along with smaller quantities of d_1 (18%), d_3 (18%), d_4 (11%) and d_5 (5%) material.

The mass spectrometric results (see Table I) with the 3,3-d_2 (XXV) and 1,1,3,3-d_4 (XXIV) 5α-androstan-11-one analogs show that virtually no hydrogen transfer occurs from C-1 to the most abundant fragment ion (*m/e* 164 in Fig. 1) and that the mechanism implied by the arrows in I cannot be operative for it. In view of this rather surprising result, it was decided to label 5α-androstan-11-one (IX) with deuterium at as many other nuclear positions as possible, in the hope of gaining some knowledge of the fragmentation processes. There follows an account of the chemical experiments carried out to label the following nuclear carbon atoms: 2, 4, 5, 6, 8, 9, 12 and 17. Considering that positions 1 and 3 have already been labeled (XV, XXIV, XXV), this means that all nuclear positions in rings A, B and C, except for C-7, have been tagged as well as one of the ring D carbon atoms.

Labeling at C-2 was effected in a manner analogous to the preparation of the 3,3-d_2-ketone XXV, namely, by desulfurization of the 2-monoethylene thioketal XXVI of 5α-androstane-2,11-dione with a Raney nickel catalyst containing active deuterium. The desired 2,2-d_2-5α-androstan-11-one (XXVII) was contaminated with approximately 25% of Δ^2-5α-androsten-11-one (X),[22] the mixture being separated by gas-phase chromatography.

The method employed for labeling at C-4 was based on the same principle as for C-1. Brief heating (20 min.) under reflux of 5α-androstane-3,11-dione (VII) with sodium in deuteriomethanol–deuterium oxide provided predominantly (63%) the 2,2,4,4,9,12-d_6-analog XXVIII, containing only 12% of the 2,2,4,4,9,12,12-d_7-species. Selective thioketal formations at C-3 (XX-IX), followed by desulfurization with Raney nickel catalyst and back-exchange of all enolizable positions produced 2,2,4,4-d_4-5α-androstan-11-one (XXX) of 73% isotopic purity. Since the 2,2-d_2-ketone XXVII is already available, a comparison (Table I) of the mass spectra of these two substances furnishes the necessary information about possible hydrogen transfers originating from position 4.

Introduction of a deuterium label at C-5, though proceeding by a straightforward method, was beset by some stereochemical complications. Dibromination[29] of 5α-androstane-3,11-dione (VII) in acetic acid containing hydrogen bromide gave the crude 2,4-dibromo ketone XXXI, which was subjected to the Glaxo modification[30] of the original[31] sodium iodide procedure to provide Δ^4-androstene-3,11-dione (XXXII), contaminated by VII, XI and some 1,4-diene-3,11-dione. The saturated ketone VII and the Δ^1-3-ketone XI were removed as their water-soluble bisulfite adducts[32] and the required Δ^4-3-ketone XXXII was then purified by chromatography on alumina. Catalytic deuteration, followed by back-exchange of deuterium at C-4, afforded a mixture of 5α- and 5β-d_1-androstane-3,11-dione (XXXIII), from which the C-3 oxygen atom was removed by the standard thioketal–desulfurization sequence. Crystallization from methanol provided the pure 5β-d_1-androstan-11-one (XXXIVb), while the pure 5α-d_1-isomer XXXIVa was separated from the mother liquors by gas-phase chromatography. In the above reaction sequence, a slight excess of 5β- over 5α-isomer was produced, which is surprising in view of the earliest results[33] on the catalytic hydrogenation of a Δ^4-3,11-dione such as cortisone, where the 5α-isomer represented the principal product. When the reduction of XXXII was repeated with hydrogen and the product composition analyzed by gas-phase chromatography the ratio

tion of 3,11-diketones and of 2α-bromo-3-ketones in the steroid series can be found in H. Heymann and L. F. Fieser, *J. Am. Chem. Soc.*, **73**, 5252 (1951), and E. J. Corey, *ibid.*, **75**, 4832 (1953).

(29) See R. M. Evans, G. F. H. Green, J. S. Hunt, A. G. Long, B. Mooney and G. H. Phillips, *J. Chem. Soc.*, 1529 (1958).

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(31) G. Rosenkranz, O. Mancera, J. Gatica and C. Djerassi, *J. Am. Chem. Soc.*, **72**, 4077 (1950).

(32) R. E. Counsell, P. D. Klimstra and F. B. Colton, *J. Org. Chem.*, **27**, 248 (1962).

(33) C. Djerassi, G. Rosenkranz, J. Pataki and S. Kaufmann, *J. Biol. Chem.*, **194**, 115 (1952).

TABLE I

PRINCIPAL PEAKS (m/e) AND SHIFTS[a] OBSERVED IN MASS SPECTRA OF DEUTERATED[b] 5α-ANDROSTAN-11-ONES

Cleavage (see Fig. 1)	d_0 (IX)	1α-d_1 (84%) (XV)	2,2-d_2[c] (33%) (XXVII)	3,3-d_2[c] (48%) (XXV)	1,1,3,3-d_4[c] (60%) (XXIV)	2,2,4,4-d_4[c] (73%) (XXX)	5α-d_1[c] (69%) (XXIVa)	6,6-d_2[c] (71%) (XXXVIIa)
(4)	124	124	124	124	124 125 (5%)	124 125 (19%)	124	124 125 (21%)
(4) − H (A/B)	149	150	151	151	153	153	150	
(3) + H	151	151 152 (20%)	151	151	151 152 (*ca.* 30%)	151* 152	151	
(1)	164	164	165 (15%) 164	165 (10%) 164	165 (10%) 164	164 (40%)* 165 (49%) 166 (11%)	165 (30%) 164	165 (10–35%) 164
(2) − H and (5) − H	177	177 (74%) 178 (26%)	177 (64%) 178 (21%) 179 (13%)	177 (57%) 178 (20%) 179 (15%)	177 (50%) 178 (23%) 181 (14%)	177 (30%) 178 (29%) 179 (12%) 181 (17%)	177 (48%) 178 (52%)	179 (70%)
(6) − 2H	230	231	232	232	234	234	231	232

Cleavage (see Fig. 1)	8β-d_1 (93%) (XLI)	8β,9α-d_2[c] (63%) (XL)	9α-d_1[d] (62%) (XLII)	12α-d_1[d] (53%) (XLIV)	12β-d_1 (85%) (XLVII)	9α,12α-d_2 (91%) (XLV)	9α,12,12-d_3 (91%) (XLVI)	17,17-d_2[c] (40%) (LV)
(4)	124	124	124 125 (16%)	124 (57%) 125 (43%)	124 (49%*) 125 (51%)	124 125 (>60%)	124 (*ca.* 20%) 125 (*ca.* 40%) 126 (*ca.* 40%)	126
(4) − H (A/B)	150	150*	149 (75%)	149	149	149	149	149
(3) + H	152	153	152	152	152	153	154	153
(1)	164 (81%)* 165 (19%)	165	164 (*ca.* 13%) 165	164 (15%) 165	165	166	167	166
(2) − H and (5) − H	177 (69%) 178 (31%)	177 (25%) 178 (61%) 179 (14%)	177 (31%) 178 (69%)	177 (22%) 178 (78%)	177 (26%) 178 (74%)	177 (20%) 178 (13%) 179 (67%)	177 (20%) 179 (16%) 180 (60%)	177 (20%) 179 (45%)
(6) − 2H	230* 231	231*	230* 231	230	230	230* 231	230* 231	232

[a] Blank spaces indicate that no clear assignment is possible, while asterisks denote extensive deuterium transfer, which is covered in further detail in the Discussion section. The percentages listed in the heading for any given substance always refer to the isotopic composition, although in a few instances (where Raney nickel was used—see footnote 44) not all of the deuterium may be exclusively in the indicated position. Thus, the value 71% d_2 in XXXVIIa clearly refers only (in view of the method of preparation) to the 6,6-d_2-derivative, while the 33% d_2 in XXVII represents largely, but not necessarily exclusively, the 2,2-d_2-species, since this substance was prepared by Raney nickel desulfurization. As far as the percentages of the various peak shifts in the table are concerned, all of them represent the directly observed values (uncorrected for isotopic impurities) with the exception of the monodeuterated derivatives (the spectra of which are reproduced in Fig. 2–7), where correction could generally be made for the presence of non-deuterated material. [b] Isotopic purity is given in parentheses. [c] For other isotopic species see Experimental. [d] In the plotted spectrum the contribution of the d_0-species is subtracted.

of 5β- to 5α-isomer was found to be 1.2:1. Whether the difference between these results and the earlier ones in the cortisone series[33] is due to the extra substituent at C-17 or to differences in the catalyst (palladium–charcoal *vs.* palladium–barium sulfate[33]) was not investigated.

The availability of the Δ⁴-3-ketone XXXII also opened a route to deuterium introduction at position 6. The enolizable hydrogen atoms of Δ⁴-androstene-3,11-dione (XXXII) were replaced by deuterium, giving a mixture of 2,2,4,6,6,9,12-d_7- and 2,2,4,6,6,9,12,12-d_8-analogs (XXXV), which was hydrogenated and all enolizable positions were back-exchanged with aqueous methanolic sodium hydroxide. The 3-keto function of the C-5 epimeric mixture of 6,6-d_2-androstane-3,11-diones (XXXVI) was removed through the 3-monothioketal and the final product mixture resolved through crystallization and gas-phase chromatography into the stereochemically pure 6,6-d_2-5α- (XXXVIIa) and 6,6-d_2-5β- (XXXVIIb) androstan-11-ones.

No suitable intermediate was available for placing deuterium into position 7 and attention, therefore, was directed at methods of introducing deuterium selectively into ring C. Bromination of 11-keto steroids is known[34] to occur first at C-9 and that this also applied to the direct bromination product (XXXVIII) of 5α-androstan-11-one (IX) was confirmed in the present instance through the strong positive Cotton effect, typical of the optical rotary dispersion behavior[35] of 9α-bromo-11-keto steroids. Dehydrobromination with calcium carbonate[26] in dimethylacetamide solution led to Δ⁸-5α-androsten-11-one (XXXIX), which represented the key intermediate, since earlier work[36] had shown that reduction of Δ⁸-11-keto steroids with lithium in liquid ammonia gives steroids with the natural configuration at C-8 (β) and C-9 (α). In the present case, the reduction was carried out using deuterioammonia, generated[37] by the addition of deuterium oxide to magnesium nitride. The reaction was then carried out in the standard manner, the blue color being finally destroyed by the addition of deuteriomethanol. The resulting 11α-alcohol was directly oxidized with chromium trioxide to 8β,9α-d_2-5α-androstan-11-one (XL) and after equilibration with aqueous methanolic sodium hydroxide, there was obtained 8β-d_1-5α-androstan-11-one (XLI) of 93% isotopic purity.

The availability of 9α-bromo-5α-androstan-11-one (XXXVIII) offered a direct route to 9α-d_1-5α-androstan-11-one (XLII) by debromination[38] with zinc in deuterioacetic acid. Since the product consisted of 62% d_1- and 38% d_0-species, it was only necessary to subtract the appropriate percentage of the spectrum of

(34) H. B. Henbest, E. R. H. Jones, A. A. Wagland and T. I. Wrigley, *J. Chem. Soc.*, 2477 (1955).

(35) C. Djerassi, J. Osiecki, R. Riniker and B. Riniker, *J. Am. Chem. Soc.*, **80**, 1216 (1958).

(36) F. Sondheimer, O. Mancera, G. Rosenkranz and C. Djerassi, *ibid.*, **75**, 1282 (1953).

(37) See N. V. Sidgwick, "The Chemical Elements and their Compounds," Oxford University Press, Oxford, 1960, p. 234.

(38) See E. J. Corey and R. A. Sneen, *J. Am. Chem. Soc.*, **78**, 6269 (1956).

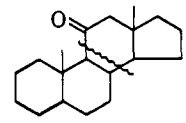
5α-androstan-11-one (IX) in order to secure the spectrum (Fig. 4) of the completely deuterated 9α-d_1-11-ketone XLII. An alternate route to the same ketone was available *via* acid-catalyzed enolization of 5α-androstan-11-one (IX) with deuterium bromide in deuterioacetic acid. In practice, the isomerization was effected by a 1% solution of hydrogen bromide in deuterioacetic acid, the ratio of protons to deuterons in such a solution being less than 1:100 at equilibrium. The rate of the acid-catalyzed exchange was followed by removing aliquots from the reaction medium at various time intervals and determining the deuterium content by mass spectrometry. After forty-four hours at room temperature, there was obtained material containing 16% of d_0, 76% of 9α-d_1 (XLII) and 8% of d_2 components, the position of the introduced deuterium atom being confirmed by comparing the mass spectrum of this material with that of the specimen obtained by zinc–deuterioacetic acid debromination of the 9α-bromo-11-ketone XXXVIII.

Introduction of a deuterium label at C-12 is especially interesting since it has a bearing on the course and direction of enolization of 11-keto steroids in general. It is known[34,39] that 9α-bromo-11-keto steroids can be isomerized with hydrogen bromide to the 12α-bromide and a specimen of 12α-bromo-5α-androstan-11-one (XLIII) thus prepared had the expected[35] negative Cotton effect associated with an axial bromine atom in that position. Reduction with zinc in deuterioacetic acid furnished 12α-d_1-5α-androstan-11-one (XLIV) of 53% isotopic purity. Since the remainder represented unlabeled 5α-androstan-11-one (IX), a simple subtraction of the two mass spectra gave the mass spectrum of the pure 12α-d_1-11-ketone XLIV.

As mentioned above (see XXII, XXVIII, XXXV), it would appear that replacement by deuterium of two of the enolizable hydrogens adjacent to the 11-keto function can be effected readily by base catalysis, while introduction of a third deuterium atom can be accomplished only with difficulty. That this is indeed the case was shown by heating under reflux (20 min.) a 1% solution of 5α-androstan-11-one (IX) in deuteriomethanol solution containing 20% of deuterium oxide and 2% by weight of sodium, a dideuterio ketone of 91% purity being obtained, contaminated by 2% of d_1- and 7% of d_3-species. In view of the fact that steroidal 11-ketones are initially brominated[34] at C-9 under the influence of acid catalysis, it is reasonable to assume that enolization to C-9 is most facile. Furthermore, Corey and Sneen[38] have shown that axial enolization and ketonization occur in preference to equatorial enolization and ketonization, the magnitude of this effect depending upon the solvent. Since, as will be seen below, it can be demonstrated by mass spectrometry that this dideuterated material is a 9,12-d_2-5α-androstan-11-one, its stereochemistry (XLV) (9α,12α-d_2) is as rigidly defined as that of 12α-d_1-5α-androstan-11-one (XLIV), obtained by zinc–deuterioacetic acid debromination of the 12α-bromo-11-ketone XLIII, since both stereochemical assignments at C-12 depend on axial ketonization of the same enol.

Prolonged heating (four days) under reflux of 5α-androstan-11-one (IX) under the above defined basic conditions gave the 9,12,12-d_3-ketone XLVI (92% d_3 and 9% d_2), which underwent preferential back-exchange of two deuterium atoms upon treatment with aqueous methanolic base for one hour at reflux temperature to lead to 12β-d_1-5α-androstan-11-one (XLVII).

The mass spectrometric proof for the *location*[39a] (but *not stereochemistry*) of the deuterium atoms in the 9α,-12α-d_2- (XLV) and 12β-d_1- (XLVII) 11-ketones is as follows. The mass spectrum (Fig. 1) of 5α-androstan-11-one contains a medium intensity peak at *m/e* 124. From an examination of the spectra (see Table I) of the many deuterated analogs, this peak can be ascribed unequivocally to the fragmentation shown schematically by the wavy line, the charge remaining with the ketone-containing moiety



As expected, in the mass spectrum of 9α-d_1-5α-androstan-11-one (XLII), prepared by either one of the above discussed methods, the *m/e* 124 peak is mainly (84%) unmoved: only 16% of the peak appears at *m/e* 125 due to cleavage mechanisms involving transfer of deuterium from C-9 to the charge-retaining fragment. The mass spectrum of the 12α-d_1-ketone XLIV shows 57% of the peak remaining at *m/e* 124, while 43% appears at *m/e* 125. Thus 57% of the 12α-deuterium atom is lost from the charge-containing fragment during the cleavage process, the mechanism of which will be discussed later.

Now if the dideuterated species were a 12,12-d_2-derivative instead of the 9α,12α-d_2-ketone XLV assumed above, this would necessitate that the substance obtained by rapid back-exchange of two deuterium atoms from the 9,12,12-d_3-analog XLVI be the 9α-d_1- (XLII) rather than the 12β-d_1- (XLVII) ketone. The mass spectrum of the product resulting from back-exchange of the 9,12,12-d_3-ketone XLVI excludes this, since the original *m/e* 124 peak is now divided between *m/e* 124 (49%) and *m/e* 125 (51%). Therefore, the monodeuterio ketone XLVII is correctly represented as a 12-d_1-11-one, whence it follows that the initial dideuterated product obtained by brief treatment of 5α-androstan-11-one (IX) with base in deuteriomethanol-deuterium oxide must be the 9α,12α-d_2-11-ketone XLV.

In the following discussion of the mass spectra reference is made to a number of other ring C-deuterated 11-ketones. The 9α,12α-d_2 (XLVIII), 9α,12,12-d_3 (XLIX) and 12β-d_1 (L) analogs of Δ²-5α-androsten-11-one (X) were prepared in the same manner as described above for the ring A saturated substances, while 9α,12α-d_2-5α-pregnan-11-one (LII) was obtained by short base-catalyzed equilibration of 5α-pregnan-11-one (LI).[16]

Labeling of ring D with deuterium was effected principally at C-17 by desulfurization of the 17-ethylene thioketal IV with deuterio-Raney nickel, followed by hydrolysis of the 3-acetate function. The mass spectrum of the crude hydroxy ketone LIII showed the incorporation of principally two (17,17-d_2) and three (16,-17,17-d_3?) deuterium atoms and the substance was transformed directly into the tosylate LIV. Elimination of the tosylate function on basic alumina[23] gave 17,17-d_2-Δ²-5α-androsten-11-one (LVI) (containing an almost equal amount of d_3-species), while lithium aluminum hydride reduction of the tosylate LIV and chromium trioxide oxidation of the intermediate 11β-alcohol led to the ring D labeled 5α-androstan-11-one (40% d_2 (LV) and 36% d_3).

Discussion of Mass Spectral Fragmentation Processes

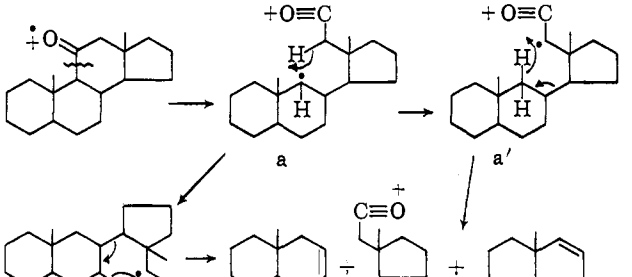
The mass spectrum of 5α-androstan-11-one (IX), first determined in our initial survey paper,[3] is reproduced in Fig. 1. Six principal fragmentation modes can

39) E. R. H. Jones and D. J. Wluka, *J. Chem. Soc.*, 907 (1959).

(39a) NOTE ADDED IN PROOF.—These assignments have now been confirmed by n.m.r. spectrometry; see footnote 27a.

be indicated unambiguously by examining the presence of absence of shifts (Table 1) incident to deuteration of the peaks marked (1)–(6) in Fig. 1 and it is interesting to note that three of these rough assignments (marked (1)–(3)) had been made earlier,[3] simply by comparing the mass spectra of 5α-androstan-11-one (IX) and 5α-pregnan-11-one (LI). The only modifications that have to be made apply to the *m/e* 149 peak and to the observation that the *m/e* 177 peak actually consists of two different fragments, the predominant one being due to cleavage (2) as suggested previously.[3] While this labeling procedure at C-17 through the additional ethyl substituent (LI) afforded some insight into the over-all fragmentation modes, only deuterium labeling can yield information on the mechanistically significant hydrogen transfers. As discussed below for each pertinent peak, every one of the fragmentations is accompanied by single or multiple hydrogen transfers; to complicate matters further, the origin of the transferred hydrogen atom is generally not limited to a single carbon atom.

Cleavage (4) (Peak *m/e* 124 in Fig. 1).—No assignment had been made previously[3] to this peak, but it is clear from an inspection of Table I that it cannot contain ring A. A two-mass unit shift to *m/e* 126 is noted in the 17,17-d_2-11-ketone LV, indicating that ring D is involved. Deuterium labels at positions 5, 6 and 8 do not cause this peak to move (Table I), thus showing that it must arise formally from cleavage of the bonds marked by the wavy line (4) in Fig. 1. As mentioned above in the discussion of the synthesis of 9- and 12-deuterated derivatives (XLII, XLIV, XLVII), the combined loss of deuterium from C-12, as indicated by the mass spectra (Fig. 2 and 3) of the 12α-d_1 (XLIV) and 12β-d_1 (XLVII) derivatives, is very close to one atom. This necessarily implies that one hydrogen atom must be gained from the carbon atoms comprising rings A and B (together with the C-19 angular methyl group) and one of the operative paths is illustrated below. Initial fission of the bond adjacent to the carbonyl group is typical of ketones,[12,13,40] and this may be followed by the presently documented transfer (a)[41] of the C-12 hydrogen atom to position 9. Back transfer of hydrogen, now known to accompany fission of the 8–14 bond, may occur with equal facility from C-9 (a′) or C-7 (a″) to produce the radical ion of *m/e* 124 together with the neutral olefin-containing rings A and B.



The operation of the above mechanistic path is supported by the observation that 16% of deuterium is transferred from C-9 to the charge-retaining fragment (as judged by the mass spectrum (Fig. 4) of 9α-d_1-5α-androstan-11-one (XLII)) and if the first step (a) is transfer of either one of the C-12 hydrogens to C-9, then 32% of the required hydrogen is returned (a′) from C-9 to C-12. While no C-7 labeled derivatives were available, it is very likely that an equivalent transfer occurs from C-7 (a″) and that the above-outlined path represents one of the fragmentation modes, though certainly not the exclusive one[42] leading to the *m/e* 124 ion. In the above scheme, the two hydrogens attached to C-12 become formally equivalent in the intermediate a, a feature which is again in accord with the experimental evidence (Fig. 2 and 3) that approximately equal amounts of the *m/e* 124 peak are shifted to *m/e* 125 in the 12α-d_1 (XLIV) and 12β-d_1 (XLVII) labeled derivatives.

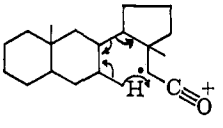
Cleavage (4) − H(Peak *m/e* 149 in Fig. 1).—The peak at *m/e* 149, originally[3] thought to be due to cleavage (3) with the additional loss of one hydrogen, is now seen to arise mainly from cleavage (4) − H, the charge remaining with rings A and B. This new assignment is established conclusively (Table I) by the spectra (*e.g.*, Fig. 7) of compounds labeled with deuterium at C-1, C-2, C-3, C-4 and C-17, while labels at C-5 and C-6 do not yield any reliable information about this fragmentation because of the close proximity of the *m/e* 151 peak (Fig. 1). The spectrum (Fig. 7) of the 1-d_1 analog XV and those of other 5α-androstan-11-ones labeled in ring A indicate that cleavage of type (3) − H is negligible.

The original assignment of a (3) − H fragmentation to the *m/e* 149 peak was strongly supported by the fact that the *m/e* 149 and 151 doublet in the spectrum (Fig. 1) of 5α-androstan-11-one (IX) moved to an *m/e* 177 and 179 doublet in the mass spectrum[3] of 5α-pregnan-11-one (LI), suggesting that both peaks contained the latter's C-17 ethyl side chain. To resolve this problem, there has now been prepared 9α,12α-d_2-5α-pregnan-11-one (LII) and its mass spectrum determined. In this spectrum (Fig. 9), the *m/e* 177 peak is unmoved, while *m/e* 179 shifts to *m/e* 181 in accordance with its assignment to cleavage (3) + H. It is established, therefore, that the *m/e* 177 peak in Fig. 9 does not arise from a (3) − H cleavage, but rather is derived from a fission of type (5) − H, the C-9 hydrogen (respectively, deuterium) being lost. The mechanism of this fragmentation will be discussed later under the appropriate (5) − H peak in the spectrum (Fig. 1) of 5α-androstan-11-one (IX).

In the spectrum (Fig. 4) of 9α-d_1-5α-androstan-11-one (XLII), the *m/e* 149 peak is mainly unmoved (Table I), calculation showing that this cleavage proceeds with 75% loss of deuterium from C-9. Examination of the mass spectra (Fig. 2 and 3) of the 12α-d_1 (XLIV) and 12β-d_1 (XLVII) 11-ketones or of the 9α,12,12-d_3 (Table I) ketone shows that no appreciable amount[43] of deuterium is transferred back from C-12 to the charge-retaining fragment. Furthermore, the mass spectrum (Fig. 5) of 8β-d_1-5α-androstan-11-one (XLI) demonstrates that deuterium is not lost from the charged ion, since the *m/e* 149 peak (Fig. 1) is now found (Fig. 5) at *m/e* 150. In the light of these facts, a plausible mechanism for this cleavage may be that shown at the top of the facing page, although participation of the C-7 hydrogen is not indispensable, since earlier studies[1] with Δ⁴-3-keto steroids have shown that fission between a double bond and a carbonyl group is feasible.

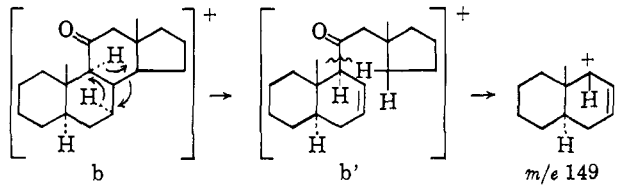
(40) A. G. Sharkey, J. L. Shultz and R. A. Friedel, *Anal. Chem.*, **28**, 934 (1956).

(41) For the sake of simplicity only, single arrows are used throughout this article. Thus α″ is meant to imply the following single electron shifts



(42) As shown in Table I, hydrogens at one or more of the positions 1, 2, 3, 4, 6 are also implicated.

(43) The *m/e* 149 peak is only moved to the same, small extent that it is in the spectrum (Fig. 4) of 9α-d_1-5α-androstan-11-one (XLII).



Cleavage (1) (Peak *m/e* 164 in Fig. 1).—This represents the most abundant fragment and the earlier conclusion[3] that it arises from rupture of the 6–7 and 9–10 bonds is completely verified by the mass spectra of the deuterated analogs (Table I). While the original studies—based as they were on mass spectral comparison of 5α-androstan-11-one (IX) and the corresponding 17-ethyl homolog LI—did not shed any light on the possible operation of hydrogen transfers, the present work shows the existence of numerous such rearrangements.

As noted in the Introduction to the present article, the original and very attractive assumption[3,14] of initial 9–10 bond cleavage through transfer of the C-1 hydrogen atom (arrows in I) is not supported by the spectrum (Fig. 7) of 1-d_1-5α-androstan-11-one (XV). Since at the time this work was done[27a] the configuration at C-1 in XV was not known with certainty[27] it was necessary to examine a 1,1-d_2-derivative and since the only available one was 1,1,3,3-d_4-5α-androstan-11-one (XXIV), we need to inspect first the mass spectrum of the 3,3-d_2-analog XXV. The peak at *m/e* 164 in the mass spectrum (Fig. 1) of 5α-androstan-11-one (IX) appeared at *m/e* 164 (90%) and *m/e* 165 (10%) in the 3,3-d_2-(XXV) spectrum[44] and the 10% shift can be attributed to one or both of two reasons: (i) some incorporation of a non-enolizable deuterium at C-7 or in rings C and D during the desulfurization process; (ii) some transfer of deuterium from C-3 to the *m/e* 164 ion during the fragmentation process. Of these possibilities, the second appears by far to be the more likely,[44] especially in view of the results cited in Table II, which shows the multiple origin of the rearranged hydrogen atoms.

TABLE II

SUMMARY OF GAIN AND LOSS OF DEUTERIUM BY *m/e* 164 FRAGMENT FROM VARIOUS CARBON ATOMS

Label on C atom not retained in *m/e* 164 ion	Atoms of D gained by *m/e* 164	Label on C atom retained in *m/e* 164 ion	Atoms of D lost by *m/e* 164
C-1	0.0	C-8	0.83
C-2	.15	C-9	0.21–0.27[a]
C-3	.10	C-12 (α)	0.15
C-4	.56	C-12 (β)	.01
C-5	0.24–0.30[45]	C-17	.0
C-6	0.16–0.44		
Total gain:	1.21–1.55	Total loss:	1.20–1.26

[a] This range is due to the uncertainty introduced by the observation that 21% of transfer was noted in the spectrum of the 9α-d_1-analog, prepared by deuterium bromide-catalyzed enolization of IX, and 27% in the spectrum of the 9α-d_1-analog, isolated in the zinc–deuterioacetic acid debromination of XXXVIII.

(44) It will be recalled that the 3,3-d_2-derivative XXV was prepared by desulfurization of the thioketal VIII with deuterium-containing Raney nickel catalyst—a process which is known (ref. 12) to proceed with scattering of deuterium. In addition to 48% of the desired d_2-analog, the material was contaminated with 18% d_1, 18% d_3, 11% d_5 and 5% d_6 species. The mass spectral analysis shows that this deuterium scattering has occurred only in that portion of molecule (essentially ring A) not contained in the *m/e* 164 fragment. This conclusion is supported in an even more striking manner by the mass spectrum of a Δ²-5α-androsten-11-one (X) sample, which had been obtained as a side product in the desulfurization of the mercaptal (XXVI) with deuterium-containing W-7 Raney nickel catalyst and which consisted of the following deuterated species: 8% d_0, 28% d_1, 33% d_2, 19% d_3, 7% d_4, 1% d_5, 3% d_6 and 1% d_7. Nearly all of the deuterium was shown to be retained between carbons 1 and 4, since the important retro-Diels–Alder peak (*m/e* 218 in Fig. 8) remained virtually unchanged at *m/e* 218 in the spectrum of the deuterated derivative.

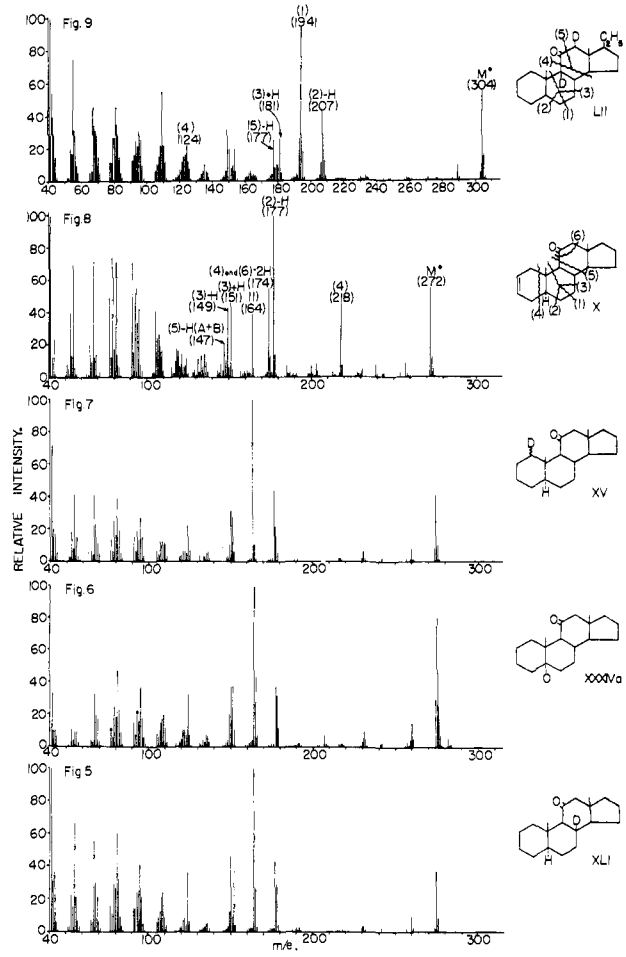


Fig. 5.—Mass spectrum of 8β-d_1-5α-androstan-11-one (XLI).
Fig. 6.—Mass spectrum of 5α-d_1-androstan-11-one (XXXIVa).
Fig. 7.—Mass spectrum of 1α-d_1-5α-androstan-11-one (XV).
Fig. 8.—Mass spectrum of Δ²-5α-androsten-11-one (X).
Fig. 9.—Mass spectrum of 9α,12α-d_2-5α-pregnan-11-one (LII).

Since the mass spectrum of the 1,1,3,3-d_4-5α-androstan-11-one (XXIV) likewise shows (Table I) only a 10% shift from *m/e* 164 to *m/e* 165 relative to the non-deuterated 11-ketone IX, we can conclude that no transfer of deuterium occurs from C-1 to the *m/e* 164 fragment and that only C-3 is implicated in the observed 10% rearrangement.

Similar reasoning[44] applied to the desulfurization (with deuterium-containing Raney nickel catalyst) of the 2-thioketal XXVI leads to the conclusion that 15% of deuterium from C-2 in 2,2-d_2-5α-androstan-11-one (XXVII) rearranged to the *m/e* 164 fragment. In the spectrum of the 2,2,4,4-d_4-11-ketone XXIV, the *m/e* 164 peak is split (Table I) as: *m/e* 164 (40%), *m/e* 165 (49%), *m/e* 166 (11%). Thus, the total fraction of deuterium transferred from C-2 and C-4 to the charge-retaining fragment is 0.71 atom (0.49 + 2 × 0.11). Hence the unexpected conclusion arises that no less than 0.56 atom of deuterium (0.71 − 0.15) is transferred from C-4.

The spectrum (Fig. 6) of 5α-d_1-androstan-11-one (XXXIVa) shows that there is also a transfer from the 5α-position to the *m/e* 164 ion, the amount ranging between 24 and 30%.[45] Again, the *m/e* 164 peak is partially shifted (Table I) to *m/e* 165 in the spectrum of 6,6-d_2-5α-androstan-11-one (XXXVIIa), the extent

(45) The 5α-d_1-analog contains 7% of d_2- and 2% of d_3-species. If it is assumed that the deuterium in these species is not retained at all in the *m/e* 164 fragment, then one arrrives at a 30% transfer figure. Alternatively, if it is assumed that the extra deuterium is completely attached to carbon atoms contained in the *m/e* 164 ion, then the lower figure (24%) follows.

of this hydrogen transfer being in the range of 10–34%, depending upon the assumption about the location of the deuterium in the 25% of contaminating d_3-species in XXXVIIa.

From the above calculations, it can be seen that the gain of deuterium by the *m/e* 164 fragment from carbon atoms 1, 2, 3, 4, 5 and 6 lies in the range of 1.2–1.55 atoms (summarized in Table II). The validity of these calculations can be tested by labeling with deuterium the carbon atoms retained in the *m/e* 164 ion, since it is absolutely necessary that an equal amount of back-transfer (or loss) of deuterium should occur from this portion of the molecule. Since seven derivatives of 5α-androstan-11-one (IX) were available in which one or more of the carbon atoms 8, 9, 12 and 17 were labeled with deuterium, calculations similar to the ones just discussed lead to the situation summarized in Table II, namely, that approximately 1.20–1.26 atoms of deuterium are back-transferred from those carbon atoms[46] of the *m/e* 164 fragment.

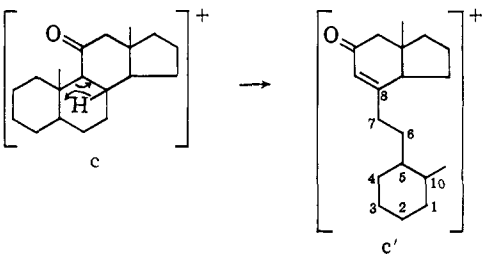
Since all carbon atoms, except for C-19, which can possibly lose hydrogen or deuterium to the *m/e* 164 fragment, have been fully labeled, while of those which can lose hydrogen or deuterium from this fragment C-7, C-14, C-15, C-16 and C-18 have not been so marked, one would expect the accounted gain to be greater than the accounted loss. This is precisely the conclusion derived from Table II. Furthermore, since the amount of transfer occurring is in excess of one atom in each direction (Table II), then an appreciable amount of the cleavage is probably taking place through transfer of two hydrogens in *each* direction. Possible mechanism for this cleavage will be considered below together with the discussion of the related cleavages (3) + H and (2) − H (peaks *m/e* 151 and 177 in Fig. 1).

Cleavages (3) + H and (2) − H (Peaks *m/e* 151 and 177 in Fig. 1).—That the *m/e* 151 and *m/e* 177 (for further discussion see cleavage (5) − H) peaks are correctly attributed to cleavages (3) + H and (2) − H, respectively, is established clearly by the deuterium labeling experiments summarized in Table I. In contrast to the situation occurring with the base peak *m/e* 164 (Fig. 1), a quantitative estimate of the hydrogen transfers involved from various carbon atoms is not possible for either of these fragments for two reasons. First, the reduced intensities of these two peaks relative to the *m/e* 164 base peak make such estimates less secure. Second, the close proximity of the *m/e* 149 and 151 peaks on the one hand, and the composite nature (*vide infra*) of the *m/e* 177 peak on the other, makes such estimates less reliable. Nevertheless, the following generalizations are possible: (i) Transfer from C-4 is very important (to the extent of *ca.* 50%) in both cleavages (3) + H and (2) − H, as it was in cleavage (1)—see Table I.

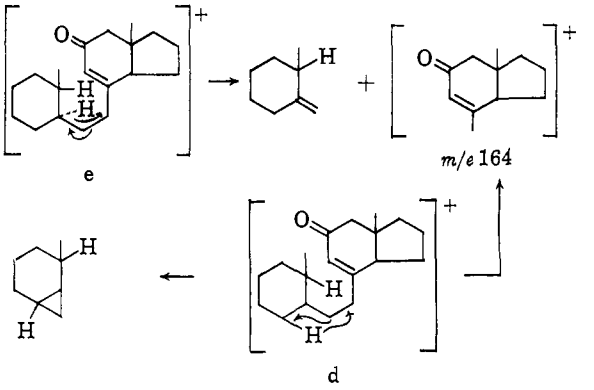
(ii) Just as in the case of cleavage (1), cleavage (2) − H occurs with approximately 80% loss of hydrogen from C-8 (see Table I and Fig. 5, the *m/e* 177 peak remaining unchanged), in contrast to the (3) + H cleavage, where the C-8 hydrogen is retained in the *m/e* 151 ion, since a shift to *m/e* 152 is encountered (Table I and Fig. 5) upon deuteration at C-8. This suggests that the first process in the formation of the *m/e* 164 and 177 ions may be the one depicted by c, the product taking up the most stable conformation c′.

In the intermediate c′, fission of the 7–8 bond would not be expected to be favored and this is in agreement

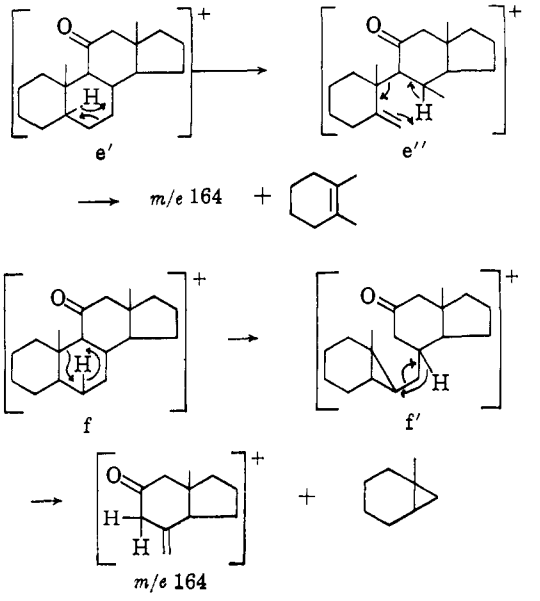
(46) It is interesting to note that those species which cleave with loss of deuterium from C-9 do not seem to lose deuterium from C-12, and *vice versa*, since the spectrum of 9,12,12-d_3-5α-androstan-11-one (XLVI) shows negligible peaks at *m/e* 164 and 165, virtually complete shift to *m/e* 166 and 167 having occurred.



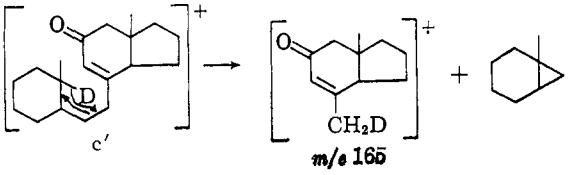
with the observation that none of the *m/e* 151 peak (cleavage (3) in Fig. 1) is formed by this mechanism. During the rupture of the 6–7 bond of c′, transfer of hydrogen from one of the following carbon atoms C 1, 2, 3, 4, 5, 6, 10[47] or 19 must occur since the base peak is found at *m/e* 164 rather than at *m/e* 163. Because of the spatial arrangement, it can be seen that abstraction from C-4 (arrows[41] in d), C-10,[47] C-5 (arrows in e) and C-6 will be more likely than from C-1, C-2 or C-3, in agreement with the results summarized in Table II.



The sequence of hydrogen transfers is, of course, not established and the rearrangement of the C-5 hydrogen

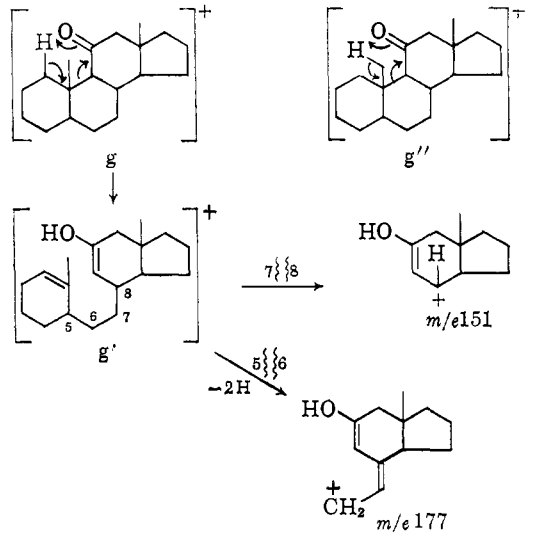


(47) In the intermediate c′, back-tansfer of the original C-8 hydrogen from position 10 is obviously feasible and thus accounts for the fact that about 20% of the *m/e* 164 peak moved (see Table I) to *m/e* 165 in the 8β-d_1-11-ketone XLI.



may also precede the C-8 hydrogen transfer as indicated by the arrows[41] in e′ and e″. Similarly, the rearrangement of the C-6 hydrogen may be visualized through the alternate sequence f–f′.

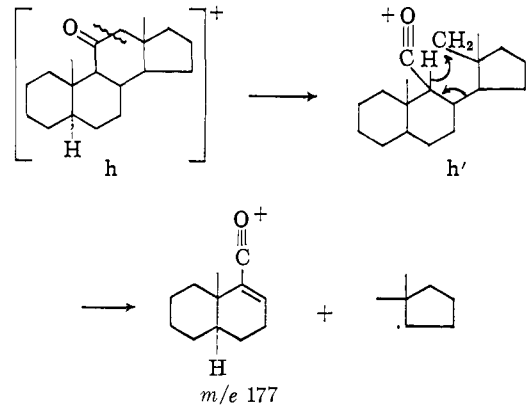
Examination of the *m/e* 151 and 177 peaks in the spectra (see Table I) of 3,3-d_2- (XXV) and 1,1,3,3-d_4- (XXIV) 5α-androstan-11-one shows that deuterium is transferred from C-1 to the extent of about 30% (±10%), since partial movements to *m/e* 152 and 178 are noted. These observations show that the originally postulated[3,14] mechanism (see arrows in I and g) is at least partly operative insofar as the satellite peaks *m/e* 151 and *m/e* 177 are concerned, fission of the allylically activated 7–8 bond in g′ leading to the *m/e* 151 ion, while rupture of the other activated bond (5–6) as well as loss of two hydrogen atoms gives the *m/e* 177 ion. Furthermore, it is possible[14] that the hydrogen atoms of the angular methyl group are implicated (g″), but labels at the rather inaccessible 7- and 19-positions would be necessary to substantiate some of these tentative, mechanistic proposals.



Cleavage (5) − H (Peak *m/e* 177 in Fig. 1).—In the mass spectrum (Fig. 7) of 1α-d_1-5α-androstan-11-one (XV), about 26% of the *m/e* 177 peak is moved to *m/e* 178. This can be interpreted either as a transfer of the C-1 hydrogen atom and/or to retention in a second fragment ion, which also corresponds to mass 177. That the latter process is partly operative is demonstrated by the mass spectral shifts (Table I) to *m/e* 181 in the ring-A labeled 1,1,3,3-d_4 (XXIV) and 2,2,4,4-d_4 (XXX) analogs, which occurred to about the same extent and which demonstrate that approximately 20% of the *m/e* 177 peak is due to the ion arising from cleavage (5) − H. It has already been noted above from a comparison of the mass spectra of 5α-pregnan-11-one (LI) and its 9α,12α-d_2-derivative (LII) (Fig. 9) that the hydrogen atom attached to C-9 is the one that is transferred and this conclusion is verified by the observation (Table I) that *ca.* 20% of the *m/e* 177 peak remains unmoved in the mass spectra of the 9α,12α-d_2- (XLV) and 9α,12,12-d_3- (XLVI) 5α-androstan-11-ones.

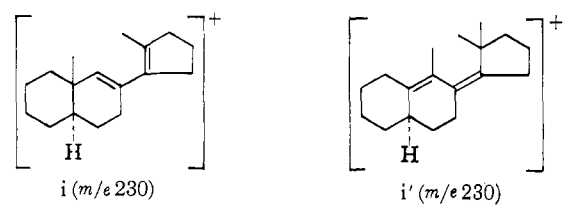
It seems reasonable to envisage this cleavage as arising through homolytic fission of the 11–12 bond (wavy line in h) adjacent to the carbonyl group,[12,13,40] followed by radical abstraction[41] from C-9 (h′) and associated cleavage of the 8–14 linkage.

Cleavage (6) − 2H (Peak *m/e* 230 in Fig. 1).—This peak, though of low intensity, is most interesting, since the multiple hydrogen rearrangements involved in its formation can be defined from an inspection of



the various available deuterated 5α-androstan-11-ones. Movement to *m/e* 231 in the spectrum (Fig. 7) of 1-d_1-5α-androstan-11-one (XV) indicates the retention of ring A. Indeed, that the *m/e* 230 peak (Fig. 1) does encompass rings A, B and D is clearly indicated (Table I) by the spectra of the 1,1,3,3-d_4 (XXIV), 2,2,4,4-d_4 (XXX), 5α-d_1 (XXXIVa), 6,6-d_2 (XXXVIIa) and 17,17-d_2 (LV) labeled 5α-androstan-11-ones. It must, therefore, be ascribed to cleavage (6) − 2H.

Analysis of the spectra of the labeled derivatives indicates that no noticeable[48] loss of hydrogen occurs from positions 1, 2, 3, 4, 5, 6 and 17, but that appreciable (*ca.* 0.5–0.6 atom) loss of hydrogen takes place from C-8 (Fig. 5) and C-9 (Fig. 4). The spectra (Fig. 2 and 3) of 12α-d_1- (XLIV) and 12β-d_1- (XLVII) 5α-androstan-11-one show clearly that there is no appreciable back-transfer of hydrogen from C-12 to the charge-retaining fragment. This is in contrast with cleavage (4) (peak *m/e* 124), in which hydrogen from C-12 is transferred (see a) to C–9, presumably with development of radical character at C-12 (see a′). Such a radical would make fission of the 12–13 bond unfavorable and this appears to be the reason for lack of transfer from C-12 in the present instance. The most reasonable mechanism for this cleavage seems to be initial rupture of the 9–11 bond (see discussion of *m/e* 124 ion), followed by homolysis of the 12–13 linkage accompanied by loss of two hydrogen atoms (mainly from C-8 and C-9), or homolysis of the 12–13 bond accompanied by loss of one hydrogen and then succeeded by expulsion of a second one. The *m/e* 230 ion is presumably represented by the diene i (minus one electron) or possibly the rearranged diene i′.



The Mass Spectrum (Fig. 8) of Δ²-5α-Androsten-11-one (X).—The fragmentation pattern of Δ²-5α-androsten-11-one (X), as deduced from the mass spectra of several deuterated analogs, is represented by the wavy lines marked (1)–(6) in Fig. 8. The peaks at *m/e* 149, 151, 164, 177 and 218 move by two mass units in both the spectra (see Table III) of 9α,12α-d_2- (XLVIII) and 17,17-d_2- (LVI) Δ²-5α-androsten-11-ones and do not contain ring A as demonstrated by labeling[44] with deuterium in ring A. The peaks at *m/e* 218 and 174 do not occur in the mass spectrum (Fig. 1) of the

(48) It is not possible to calculate exact figures due to the relatively low abundance of the *m/e* 230 ion and the presence of similar low intensity peaks in its proximity, but qualitative conclusions are easily feasible.

TABLE III

PRINCIPAL PEAKS (m/e) AND SHIFTS OBSERVED IN MASS SPECTRA OF DEUTERATED[a] Δ^2-5α-ANDROSTEN-11-ONES

Cleavage[b]	d_0 (X)	9α,12α-d_2 (88%)[c] (XLVIII)	12β-d_1 (76%)[b] (L)	9,12,12-d_3 (72%)[b] (XLIX)	17,17-d_2 (40%)[b] (LV)
(5) − H (A + B)	147	147 (80%), 148 (20%)	147	147 (80%), 148 (20%)	147
(3) − H	149	151	150	152	151
(3) + H	151	153	152	154	153
(1)	164	166	165	167	166
(4) + (6) − 2H	174	175	174	175	176
(2) − H	177	179	178	180	179
(4)	218	220	219	221	220

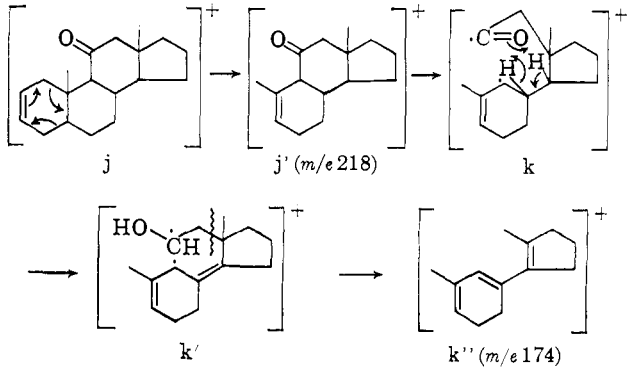
[a] Isotopic purity is given in parentheses. [b] See Fig. 8. [c] For other isotopic species see Experimental.

saturated 5α-androstan-11-one (IX) and can be ascribed, therefore, to the presence of the Δ^2-double bond.

The m/e 218 peak (j') arises from retro-Diels–Alder reaction of ring A (arrows in j) with expulsion of butadiene,[49] while the m/e 174 represents the further loss of 44 mass units, analogous to cleavage (6) − 2H in 5α-androstan-11-one (IX) itself (Fig. 1). This conclusion is established (Table III) by the observation that both hydrogens at C-17 are retained (LVI) while both hydrogens at C-12 are lost (XLVIII–L). In the corresponding fragmentation (*i.e.*, (6) − 2H) in the mass spectrum (Fig. 1) of 5α-androstan-11-one (IX), deuterium (0.5–0.6 atom) is lost from C-9, whereas in the present case (XLVIII and XLIX in Table III) none is lost from that position.

Presumably, homolytic cleavage of the 9–11 bond is an important primary process in this cleavage of the saturated 11-ketone IX, but since the radical formed at C-9 is not a particularly favorable one, subsequent quenching of it (by hydrogen or methyl migration) will be a common process. Further transfer of hydrogen from C-9 is then theoretically tenable and does, in fact, occur. In the (6) − 2H cleavage from Δ^2-5α-androsten-11-one (X), one would expect cleavage of the 9–11 bond in the retro-Diels-Alder fragment j' to be facilitated, since any radical or ion character developed at C-9 (see k) will be stabilized by the allylic 5–10 double bond, thus explaining the much greater abundance of the (6) − 2H cleavage relative to the corresponding fragmentation in the saturated series.

Furthermore, as the radical k at C-9 is relatively stable, its tendency to gain a hydrogen radical and subsequently to become a source of hydrogen being transferred from the charge-retaining fragment will be considerably reduced, exactly as is found. If the two hydrogens being lost originate from C-8 and C-14 (k → k') and if the hydrogen transfer process is followed by homolysis of the 12–13 bond (wavy line in k'), a stable triene (k'') of mass m/e 174 results.



The cleavage (5) − H, giving rise to the m/e 147 peak (charge remaining with rings A and B) in Fig. 8 occurs (Table III) with approximately 80% loss of deuterium from C-9 and no transfer of deuterium from C-12 to the charge-retaining fragment of the appropriately labeled substrates, exactly as was found for the corresponding cleavage in 5α-androstan-11-one (IX).

No comment is required for the remaining peaks in Fig. 8, which are more or less analogous to those observed (Fig. 1) in 5α-androstan-11-one. Similarly, the mass spectra (not reproduced) of the two labeled 5β-androstan-11-ones XXXIVb and XXXVIIb completely confirm the earlier conclusion[3] that the 5α- and 5β-androstan-11-ones resemble each other closely in their principal modes of fragmentation and differ only in the intensity relationship of the various peaks.

General Conclusions

Just as in the earlier mass spectrometric studies[12,13] with bicyclic model compounds of the α- and β-decalone series, the present investigation of labeled analogs of a steroidal 11-ketone demonstrates the occurrence of extensive hydrogen rearrangements accompanying the principal fragmentation processes. The bicyclic substances represent better models for steroids oxygenated in rings A and B, rather than in ring C, as will be shown in subsequent articles dealing with such deuterated ring A keto steroids, but certain similarities do emerge, notably the expected[40] cleavage of the bond adjacent to the carbonyl group, which directs many of the subsequent cleavage processes.

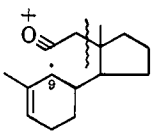
It is obvious from the present work that a detailed knowledge of the mass spectral fragmentation patterns of such polycyclic ketones is best studied through extensive deuterium labeling of a few selected test cases and future articles with 3-, 7-, 12-, 16- and 20-keto steroids will illustrate this point. While some of the hydrogen rearrangements uncovered in this work appear to proceed in a somewhat random manner (*e.g.*, Table II), there is no question that mechanistically reasonable deductions arise. A general survey of these deductions is best deferred until the results from all of the model steroid studies are available, but the parallel recapitulation of three fragmentation processes shown at the top of the facing page seems appropriate and emphasizes the feasibility of the ultimate aim of this work, namely, the *a priori* qualitative prediction of major fragmentation patterns of given molecular structures.

Experimental[50]

5α-Androstane-11,17-dione-3β-ol Acetate (III).—A solution of 10 g. of Δ^{16}-5α-pregnene-11,20-dione-3β-ol acetate (II)[19] and 2.8

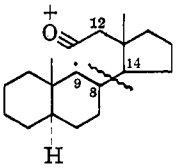
(49) A completely analogous fragmentation has been noted (H. Ziffer and U. Weiss, *J. Org. Chem.*, **27**, 2694 (1962)) in the mass spectrum of the bicyclic 9-methyl-*trans*-1,4,9,10-tetrahydronaphthalene, peaks corresponding to toluene and butadiene being observed.

(50) All melting points are corrected and were determined in capillaries. Rotations were measured in chloroform and ultraviolet absorption spectra in 95% ethanol solution. The optical rotatory dispersion curves were obtained by Mrs. Ruth Records using a Nippon Bunko (Japan Spectroscopic Manufacturing Co., Ltd.) automatically recording spectropolarimeter (model ORD-2). Thin-layer chromatography (T.L.C.) was performed on silica gel G (E. Merck, A.G., Darmstadt), the spots being developed by spraying with a 2% solution of ceric sulfate in 2 *N* sulfuric acid and subsequent heating. All mass spectra were determined with a Consolidated Electrodynamics Corp. mass spectrometer No. 21-103C using an all-glass inlet system heated to 200°, while the isatron temperature was maintained at 270°. The ionizing energy was kept at 70 e.v and the ionizing current at 50 μa. All microanalyses were carried out by Messrs. E. Meier and J. Consul.



m/e 174 in spectrum (Fig. 8) of X

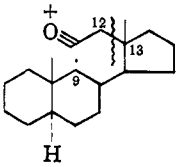
(i) Relatively stable radical generated at C-9, hence no great tendency toward migration to C-9 and no consequent hydrogen transfer from C-9



m/e 124 in spectrum (Fig. 1) of IX

(i) Relatively unstable radical at C-9, hence migration to C-9 likely, especially from the sterically most favored C-12 position

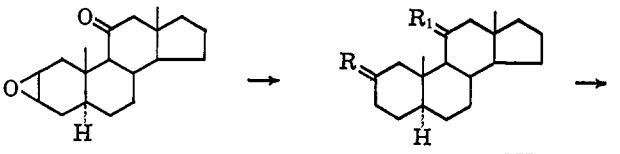
(ii) Subsequent loss of hydrogen from C-9 now possible and may be favored, especially since accompanying homolytic fission of 8–14 bond results in formation of 8–9 double bond



m/e 230 in spectrum (Fig. 1) of IX

(i) Migration to quench relatively unstable C-9 radical likely, but not from C-12, because of necessity for subsequent 12–13 bond fission

(ii) Since two hydrogen atoms must be lost, involvement of the sterically favorable C-9 hydrogen is a likely process



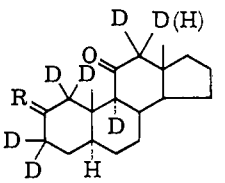
XIX

XX, R = R$_1$ = $\underline{\text{OH}}$H

XXI, R = R$_1$ = O

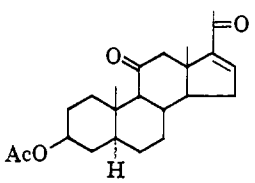
XXVI, R = $\langle^{S}_{S}]$; R$_1$ = O

XXVII, R = D$_2$; R$_1$ = O

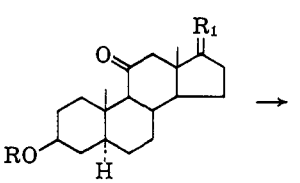


XXII, R = O

XXIII, R = $\langle^{S}_{S}]$



II

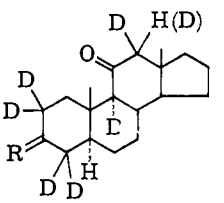


III, R = Ac; R$_1$ = O

IV, R = Ac; R$_1$ = $\langle^{S}_{S}]$

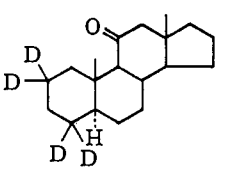
V, R = H; R$_1$ = H$_2$

VI, R = Ts; R$_1$ = H

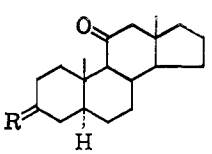


XXVIII, R = O

XXIX, R = $\langle^{S}_{S}]$



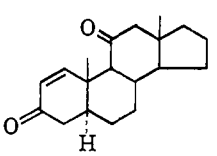
XXX



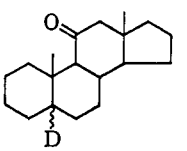
VII, R = O

VIII, R = $\langle^{S}_{S}]$

IX, R = H$_2$

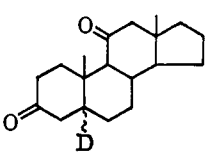


XI

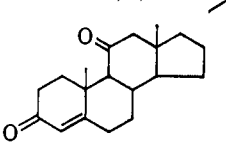


XXXIVa, 5αD

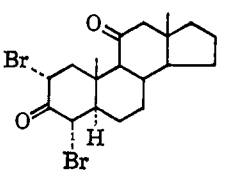
b, 5βD



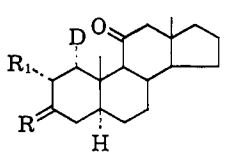
XXXIII



XXXII



XXXI

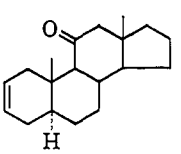


XII, R = O; R$_1$ = D

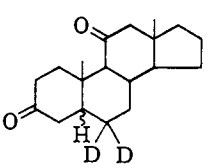
XIII, R = O; R$_1$ = H

XIV, R = $\langle^{S}_{S}]$; R$_1$ = H

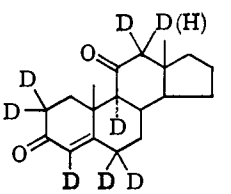
XV, R = H$_2$; R$_1$ = H



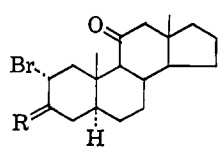
X



XXXVI



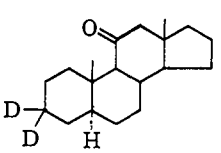
XXXV



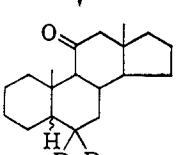
XVI, R = O

XVII, R = $\underline{\text{OH}}$H

XVIII, R = $\underline{\text{OH}}$H

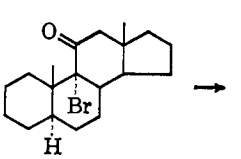


XXV

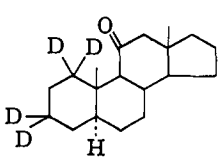


XXXVIIa, 5αH

b, 5βH

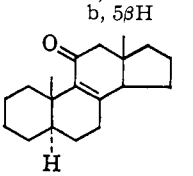


XXXVIII

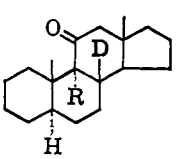


XXIV

(XXIII)

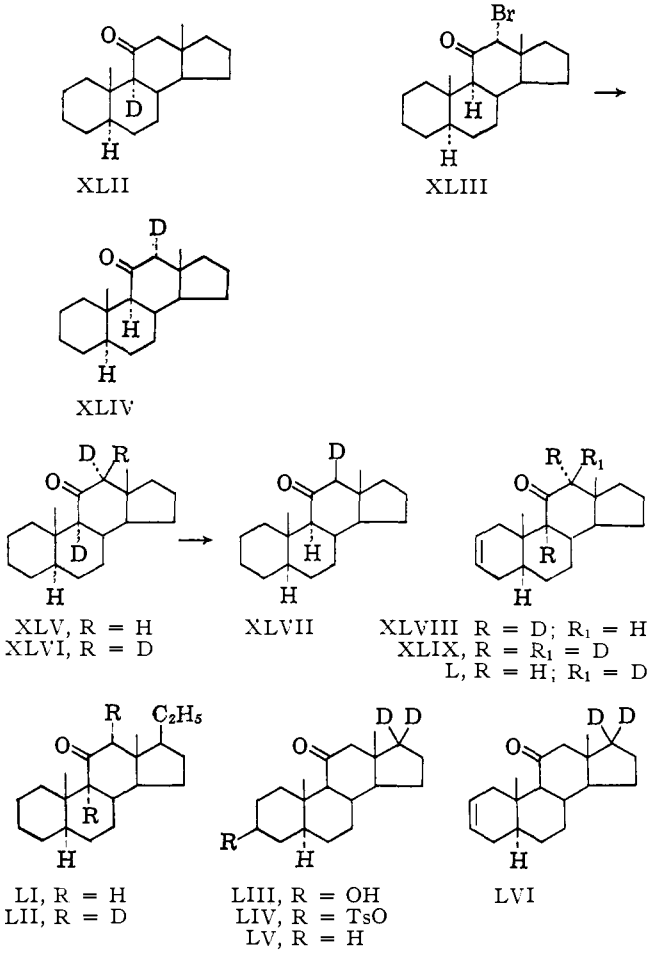


XXXIX



XL, R = D

XLI, R = H



XLII XLIII XLIV

XLV, R = H
XLVI, R = D

XLVII

XLVIII R = D; R_1 = H
XLIX, R = R_1 = D
L, R = H; R_1 = D

LI, R = H
LII, R = D

LIII, R = OH
LIV, R = TsO
LV, R = H

LVI

g. of hydroxylamine hydrochloride in absolute ethanol (50 cc.)-pyridine (14 cc.) was heated under reflux for 35 min. Water was then added until the solution was just homogeneous when hot. On slow cooling to −16°, the oxime (8.6 g., m.p. 214–223°) crystallized out. The analytical sample was obtained after one recrystallization from methanol; m.p. 217–222°, $[\alpha]_D$ +48° (*c* 1.6), λ_{max}^{Nujol} 5.82 and 5.88 μ, λ_{max} 234 mμ (ε 16,100).

Anal. Calcd. for $C_{23}H_{33}NO_4$: C, 71.29; H, 8.58; N, 3.61. Found: C, 71.34; H, 8.43; N, 3.48.

To the above oxime (1.06 g.) in 2.8 cc. of anhydrous pyridine was added with stirring at 0° a solution of *p*-acetamidobenzenesulfonyl chloride (1.21 g.), the temperature being maintained below 5°. The solution was stirred at that temperature for 2 hr. and then at room temperature for a further 2 hr. After pouring the mixture into ice (20 g.) and concd. sulfuric acid (6.0 cc.) and keeping it at 0° overnight, the solid was collected and washed with warm water. The infrared spectrum (chloroform) exhibited bands at 5.76, 5.81 and 5.85 μ, but no hydroxyl absorption. A thin-layer chromatogram on silica gel (developed with ether) showed only one spot, thus supporting the infrared evidence that no appreciable hydrolysis of the acetate function had occurred. The dried residue (1.26 g.) was extracted several times with ether and the solution filtered through 30 g. of alumina (Merck, neutral, activity III). Crystallization of the resulting solid (746 mg.) from acetone–hexane gave 5α-androstane-11,17-dione-3β-ol acetate (III)[51] as colorless leaflets (644 mg.), m.p. 161–163°. An analytical specimen had the same melting point and strong infrared bands (Nujol) at 5.74, 5.78, 5.88, 8.06 and 9.7 μ; $[\alpha]_D$ +98° (*c* 2.0).

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.89, H, 9.01.

5α-Androstan-3β-ol-11-one (V).—Boron trifluoride etherate (5.0 cc.) was added to a solution of 4.72 g. of 5α-andostane-11,17-dione-3β-ol acetate (III) in 5 cc. of ethanedithiol. The stirred mixture became hot and deposited a thick paste within 2 min. After being kept at room temperature for a futher 7 min., methanol (20 cc.) was added with stirring and the solid material filtered, washed with methanol and dried. The **thioketal IV** (5.32 g., m.p. 232–234°) was used directly in the next step, but a 202-mg. sample was recrystallized from ethanol–methylene chloride giving 169 mg. of plates, m.p. 232–234°, $[\alpha]_D$ −30° (*c* 1.7); λ_{max}^{Nujol} 5.77, 5.88 and 8.00 μ.

Anal. Calcd. for $C_{23}H_{34}O_3S_2$: C, 65.36; H, 8.11; S, 15.18. Found: C, 65.29; H, 8.15; S, 15.04.

A mixture of 20.0 g. of the thioketal IV and freshly prepared W-7 Raney nickel[21] (from 300 g. of alloy) in 500 cc. of 95% ethanol was heated under reflux for 5 hr. The catalyst was removed by filtration and washed well with ethanol.[52] Potassium hydroxide (50 g.) was dissolved in the filtrate, which was then kept overnight at room temperature. Most of the ethanol was removed under reduced pressure and ether (1.5 l.) and water (1 l.) were added to the residue. The aqueous phase was re-extracted with ether, the combined organic layers were washed with water, dried and evaporated to furnish a reddish solid, which was decolorized by boiling in ether–hexane solution with animal charcoal. Concentration and cooling deposited 11.5 g. of 5α-androstan-3β-ol-11-one (V), m.p. 149–151°, while the analytical specimen exhibited m.p. 153–153.5°, $[\alpha]_D$ +58° (*c* 1.8); λ_{max}^{Nujol} 2.92, 5.91 (5.85 μ in chloroform) and 9.50 μ.

Anal. Calcd. for $C_{19}H_{30}O_2$: C, 78.57; H, 10.41; mol. wt., 290.4. Found: C, 78.54; H, 10.60; mol. wt., 290 (mass spec.).

5α-Androstane-3,11-dione (VII).—A solution of 110 mg. of chromium trioxide in 0.44 cc. of 20% sulfuric acid was added dropwise at 22° to a solution of 428 mg. of 5α-androstan-3β-ol-11-one (V) in acetone (2.0 cc.). After 2 min., the mixture was diluted with ether and water, the organic phase separated, and then washed successively with 10% sodium bicarbonate solution and water. Evaporation of the dried ether extract and crystallization of the residue (419 mg.) from aqueous methanol gave the 3,11-dione VII as long needles (380 mg.), m.p. 121–121.5°, $[\alpha]_D$ +76° (*c* 1.7), λ_{max}^{Nujol} 5.87 μ.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79; mol. wt., 288.4. Found: C, 79.23; H, 9.81; mol. wt., 288 (mass spec.).

5α-Androstan-11-one (IX).—The 3,11-diketone VII (200 mg.) was transformed into the **3-monothioketal VIII** (234 mg., m.p. 144–145°) by the above-described boron trifluoride procedure.[20] Recrystallization from methylene chloride–ethanol gave the analytical sample as long, hair-like crystals, m.p. 146–147°, $[\alpha]_D$ +61° (*c* 1.8), λ_{max}^{Nujol} 5.86 μ.

Anal. Calcd. for $C_{21}H_{32}OS_2$: C, 69.16; H, 8.85; S, 17.59. Found: C, 69.32; H, 8.89; S, 17.59.

The thioketal VIII (1.1 g.), dissolved in 95% ethanol (100 cc.), was heated under reflux with fresh W-7 Raney nickel[21] (prepared from 30 g. of alloy) for 4 hr. The catalyst was removed by filtration through a broad column of alumina (50 g., neutral, activity III) and the column was washed well with benzene. Evaporation of the filtrate gave a pale yellow oil (750 mg.) which, on crystallization from aqueous methanol at −15°, furnished 656 mg. of 5α-androstan-11-one (IX), m.p. 50–51.5° (lit.[16] m.p. 49–50°, 50–52°), the mass spectrum of which is reproduced in Fig. 1.

Δ²-5α-Androsten-11-one (X).—5α-Androstan-3β-ol-11-one (V) (200 mg.) and 400 mg. of *p*-toluenesulfonyl chloride in 1 cc. of pyridine was kept at room temperature for 18 hr., water added and the product isolated by extraction with ether. Crystallization from hexane afforded 220 mg. of the **tosylate VI**, m.p. 107–115°, which consisted of two crystalline forms, separable mechanically (m.p. 108–109° and 115–116°) after recrystallization. Homogeneity of the tosylate was confirmed by T.L.C. (developed with benzene containing 25% of ether); $[\alpha]_D$ +22° (*c* 2.4); $\lambda_{max}^{CHCl_3}$ 5.86, 7.37 and 8.5 μ.

Anal. Calcd. for $C_{26}H_{36}O_4S$: C, 70.30; H, 8.15; S, 7.20. Found: C, 70.08; H, 8.02; S, 7.42.

Basic alumina (Woelm, activity I, 630 mg.) was added to a solution of the tosylate VI (55 mg.) in anhydrous benzene (0.5 cc.), the mixture kept at room temperature for 5 days with occasional shaking and then poured into a column of similar alumina (700 mg.). Elution with benzene gave 24 mg. of solid, which was recrystallized from methanol to provide 19 mg. of Δ²-5α-androsten-11-one (X), m.p. 89–91°, its mass spectrum being reproduced in Fig. 8.

Anal. Calcd. for $C_{19}H_{28}O$: C, 83.77; H, 10.36. Found: C, 83.58; H, 10.62.

When the desulfurization of the mercaptal VIII was performed with a lower substrate/catalyst ratio, a chromatographically separable mixture of 5α-androstan-11-one (IX) and Δ²-5α-androsten-11-one (X) was obtained.[22]

2α-Bromo-5α-androstane-3,11-dione (XVI).—Bromine (0.57 cc.) in glacial acetic acid (7.5 cc.) was added dropwise with vigorous stirring to a solution of androstane-3,11-dione (VII)

(51) The diketone (m.p. 162–163°, $[\alpha]^{diox}_D$ +96°) has been described by Steiger and Reichstein (ref. 16) and by J. v. Euw and T. Reichstein, *Helv. Chim. Acta*, **25**, 988 (1942), but has not been analyzed.

(52) In a preliminary small-scale experiment, T.L.C. of the product, obtained on evaporation of the ethanol, showed the presence of a mixture of 3-acetate and 3-alcohol. Chromatography and recrystallization from aqueous methanol provided 5α-androstan-3β-ol-11-one acetate as colorless needles, m.p. 87–88°, $[\alpha]_D$ +37° (*c* 1.2); λ_{max}^{Nujol} 5.81, 5.88, 8.07, 9.75 μ. *Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.81; H, 9.96.

(3.0 g.) in 45 cc. of glacial acetic acid. Precipitation of the bromo derivative was completed by addition of water (25 cc.) and the solid was collected and washed well with water; yield 3.39 g., m.p. 200–203°. Recrystallization from chloroform–methanol gave the analytical specimen, which exhibited m.p. 208–209° dec., $[\alpha]_D$ +83° (*c* 1.5), λ_{max}^{Nujol} 5.80 and 5.84 μ, the 2α-orientation of the bromine atom being confirmed by the optical rotatory dispersion curve[35]: $[\alpha]_{589}$ +89°, $[\alpha]_{308}$ +1000°, $[\alpha]_{294}$ +446° (*c* 0.112 in methanol).

Anal. Calcd. for $C_{19}H_{27}BrO_2$: C, 62.12; H, 7.41; Br, 21.75. Found: C, 61.96; H, 7.45; Br, 21.62.

Δ¹-5α-Androstene-3,11-dione (XI).—The bromo ketone XVI (137 mg.) was added portionwise to a suspension of finely powdered calcium carbonate (150 mg.) in boiling dimethylacetamide[26] (1.5 cc.). After heating for 14 min., ether and 10% hydrochloric acid were added to the cooled mixture and the organic phase washed with water, dried and evaporated. Thin-layer chromatography (using benzene containing 3% of ether) of the resulting pale yellow oil (113 mg.) showed the presence of a principal component, accompanied by an impurity with a slightly smaller R_f value, which corresponded most likely to the anticipated[32,53] Δ⁴-3-keto isomer XXXII. Chromatography on 30 g. of neutral alumina (activity III) and elution with 4:1 benzene–hexane led to 98 mg. of the Δ¹-3,11-dione XI which, upon recrystallization from pentane, gave 61 mg. of the pure ketone, m.p. 76–78°, $[\alpha]_D$ +105° (*c* 1.3), λ_{max} 227 mμ (ε 10,700), $\lambda_{max}^{CHCl_3}$ 5.86 and 5.94 μ.

Anal. Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15; mol. wt., 286.4. Found: C, 79.51; H, 9.21; mol. wt., 286 (mass spec.).

1α-*d*₁-5α-Androstane-3,11-dione (XIII).[27]—Δ¹-5α-Androstene-3,11-dione (XI, 38 mg.) and 10% palladized charcoal catalyst (13 mg.) in 2 cc. of cyclohexane was stirred under an atmosphere of deuterium gas for 4 hr. The catalyst was removed by filtration and the filtrate evaporated to yield a white, crystalline residue (37 mg.) of **1α,2α-*d*₂-5α-androstane-3,11-dione (XII)**, m.p. 120.5–121°, undepressed upon admixture with 5α-androstane-3,11-dione (VII). The mass spectrum of the product showed the following distribution of deuterium: 8% d_0, 19% d_1, 65% d_2, 7% d_3 and 1% d_4.

The entire product was kept at room temperature for 10 min. with 150 mg. of sodium hydroxide and 3 cc. of 33% aqueous methanol. A portion of the solvent was then removed at 50° until crystallization commenced, whereupon the solid was filtered and washed with 50% aqueous methanol, affording 28 mg. of **1α-*d*₁-5α-androstane-3,11-dione (XIII)**, m.p. 119–120°. Mass spectral analysis showed the presence of 13% d_0, 84% d_1 and 3% d_2-species.

1-*d*₁-5α-Androstan-11-one (XV).—The above 28-mg. sample was converted into the thioketal XIV exactly as described for the non-deuterated analog (VII → VIII) and the resulting 34 mg. (m.p. 141–142°) was desulfurized with freshly prepared W-7 Raney nickel[21] (from 2.0 g. of alloy) in ethanol (5 cc.) in the usual manner. The product was crystallized from methanol at −15° to give 11 mg. of 1-*d*₁-5α-androstan-11-one (XV), m.p. 50.5–51°, the mass spectrum (Fig. 7) of which showed the presence of 16% d_0, 80% d_1, 2% d_2 and 2% d_3-species.

2β,3β-Oxido-5α-androstan-11-one (XIX).—Sodium borohydride (40 mg.) was added to a suspension of 2α-bromo-5α-androstane-3,11-dione (XVI) in 95% ethanol (5 cc.). The solution became homogeneous after swirling for 2 min. and, after a further 3 min., the excess reagent was destroyed by the addition of glacial acetic acid. Isolation with ether provided 216 mg. of a colorless gum, which by T.L.C. (developed with benzene, containing 30% ether) contained two main products and traces of two more polar substances. Adsorption of the gum in hexane solution on 50 g. of neutral alumina (activity III) and elution with 4:1 benzene–hexane and pure benzene led to 80 mg. of material, which crystallized with difficulty from pentane to give 11 mg. of a substance, m.p. 137–144°, homogeneous by T.L.C., which was presumably 2α-bromo-5α-androstan-3α-ol-11-one (XVIII) and which was not investigated further. After elution of a mixture (13.5 mg.) by a further 50 cc. of benzene, 15% ether in benzene (100 cc.) eluted a second substance (136 mg.) which crystallized readily from chloroform–hexane, giving 2α-bromo-5α-androstan-3β-ol-11-one (XVII) (95 mg.), m.p. 128–130°, $[\alpha]_D$ +43° (*c* 1.1), λ_{max}^{Nujol} 3.04 and 5.87 μ.

Anal. Calcd. for $C_{19}H_{29}BrO_2$: C, 61.79; H, 7.91; Br, 21.60. Found: C, 62.12; H, 8.13; Br, 21.55.

The 2α,3β-bromohydrin XVII (73 mg.) and 600 mg. of potassium hydroxide were heated in isopropyl alcohol solution (10 cc.) at 55° for 20 min. in an atmosphere of nitrogen, whereupon T.L.C. demonstrated complete reaction. Isolation with ether produced an oil (50 mg.) which crystallized from aqueous methanol to give 27 mg. of **2β,3β-oxido-5α-androstan-11-one (XIX)** as needles or plates, m.p. 85–86°, $[\alpha]_D$ +92° (*c* 1.0).

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79; mol. wt., 288.4. Found: C, 79.03; H, 9.86; mol. wt., 288 (mass spec.).

5α-Androstane-2,11-dione (XXI).—Lithium aluminum hydride (0.4 g.) was added to a solution of the 2β,3β-oxide XIX (0.43 g.) in anhydrous ether (40 cc.) and the mixture kept overnight at room temperature. After processing in the usual manner, including crystallization from hexane containing a trace of methylene chloride, there was obtained 254 mg. of **5α-androstane-2β,11β-diol (XX)** as needles, melting at 172–173°, $[\alpha]_D$ +34° (*c* 1.4), which exhibited no infrared carbonyl absorption.

Anal. Calcd. for $C_{19}H_{32}O_2$: C, 78.03; H, 11.03. Found: C, 77.87; H, 11.05.

Oxidation of 232 mg. of the diol was effected at room temperature (2 min.) with 115 mg. of chromium trioxide in 20% sulfuric acid (0.46 cc.) and acetone (5 cc.). Crystallization from hexane yielded 200 mg. of prisms of **5α-androstane-2,11-dione (XXI)** showing m.p. 187–188°, $[\alpha]_D$ +96° (*c* 1.1), $\lambda_{max}^{CHCl_2}$ 5.87 μ.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79; mol. wt., 288.4. Found: C, 78.86; H, 9.76; mol. wt., 288 (mass spec.).

5α-Androstane-2,11-dione 2-Ethylene Thioketal (XXVI).—Boron trifluoride etherate (0.1 cc.) was added to a solution of 38 mg. of the 2,11-diketone XXI in 0.1 cc. of ethanedithiol. Crystals separated within 5 min. and, after a further 3 min., 75% aqueous methanol (0.5 cc.) was added and the solid filtered; yield 42 mg., m.p. 214–215°. The melting point was unchanged on recrystallization of a small sample from ethanol–methylene chloride; $[\alpha]_D$ +30° (*c* 1.0), $\lambda_{max}^{CHCl_3}$ 5.86 μ.

Anal. Calcd. for $C_{21}H_{32}OS_2$: C, 69.16; H, 8.85; S, 17.59. Found: C, 69.19; H, 8.85; S, 17.84.

1,1,3,3-*d*₄-5α-Androstan-11-one (XXIV).—A solution of 31 mg. of 5α-androstane-2,11-dione (XXI) in 3 cc. of deuteriomethanol and 1.3 cc. of deuterium oxide containing 70 mg. of dissolved sodium was heated under reflux for 28 hr. Upon cooling, there crystallized 26 mg. of the polydeuterated ketone, which by mass spectrometry consisted of 13% d_5, 38% d_6 and 49% d_7 (XXII) species.

Most of this material (24 mg.) was treated with 0.04 cc. each of ethanedithiol and boron trifluoride etherate to provide 28 mg. of the polydeuterated thioketal XXIII, m.p. 213–214°, mass spectral analysis showing the presence of 13% d_6- and 87% d_7-species. The higher proportion of d_7-species in the thioketal as compared to the precursor ketone is evidently due to back-exchange of some of the latter's deuterium (presumably at C-1 and C-3) in the mass spectrometer.[54]

This polydeuterated thioketal XXIII (14.7 mg.) was heated under reflux for 4 hr. with 3 cc. of ethanol and freshly prepared W-7 Raney nickel[21] (from 0.4 g. of alloy). The product (11.1 mg.), isolated in the usual manner, showed only one T.L.C. spot (developed with benzene containing 30% of petroleum ether), which was identical in R_f value with 5α-androstan-11-one (IX). For back-exchange of deuterium at C-9 and C-12, 4.3 mg. of the product and 150 mg. of sodium hydroxide were heated under reflux for 2 days with 70% aqueous methanol (3 cc.) and the product (3.7 mg. of oil) isolated with ether. Crystallization from aqueous methanol at −15° afforded 3.0 mg. of **1,1,3,3-*d*₄-androstan-11-one (XXIV)**, m.p. 49–50°, the mass spectrum indicating the presence of the isotopic species: 10% d_2, 27% d_3, 60% d_4 and 3% d_5.

Preparation of Deuterium-Containing Raney Nickel Catalyst.—Sodium (190 mg.) was dissolved in deuterium oxide (1.2 cc.) and Raney nickel alloy (250 mg.) was added to this solution during 8 min., the temperature of the solution being maintained at 50 ± 2°. The supernatant liquid was removed by decantation and the residue was washed with deuterium oxide (3 × 2 cc.) and then with deuteriomethanol (2 × 1 cc.). Catalysts prepared in this manner were always used immediately and washed into the reaction vessel by the deuteriomethanol in which the desulfurization was to be conducted.

3,3-*d*₂-5α-Androstan-11-one (XXV).—5α-Androstane-3,11-dione 3-ethylene thioketal (VIII) (9.0 mg.) and deuterium-containing Raney nickel (prepared from 250 mg. of alloy as described above) in 2 cc. of deuteriomethanol were heated under reflux for 1 hr., at which time reduction was complete as shown by T.L.C. analysis. The isolated product (7.0 mg.) and sodium hydroxide (20 mg.) in 3 cc. of 70% aqueous methanol were heated under reflux for 1 hr. and the resulting product crystallized from aqueous methanol; yield 1.0 mg., m.p. 49–50°; isotopic composition by mass spectrometry: 18% d_1, 48% d_2, 18% d_3, 11% d_4, 5% d_5.

2,2-*d*₂-5α-Androstan-11-one (XXVII).—5α-Androstane-2,11-dione 2-ethylene thioketal (XXVI, 8 mg.) was desulfurized as described in the preceding paragraph except that the reaction time was extended to 2 hr.; T.L.C. showed that the resulting colorless oil (5.2 mg.) consisted of two components and gas-phase chromatography showed the presence of *ca.* 25% of Δ²-11-ketone (X) as contaminant. By means of preparative gas-phase chromatography (Wilkens Aerograph instrument, using a 15% phenyldiethanolamine succinate column at 173° with a nitrogen

(53) C. Djerassi and C. R. Scholz, *J. Am. Chem. Soc.*, **69**, 2404 (1947).

(54) See footnote 7 in ref. 13.

pressure of 50 lb./sq. in.) there was obtained 5α-androstan-11-one of the isotopic composition: 12% d_1, 33% d_2 (XXVII), 16% d_3, 18% d_4, 12% d_5, 9% d_6; and Δ^2-5α-androsten-11-one consisting of 8% d_0, 28% d_1, 33% d_2, 19% d_3, 7% d_4, 1% d_5, 3% d_6 and 1% d_7, virtually all of the deuterium being located on carbon atoms 1, 2, 3 and 4.[44]

2,2,4,4-d_4-5α-Androstan-11-one (XXX).—Sodium (100 mg.) was dissolved in a mixture of 1.0 cc. of deuterium oxide and 3.0 cc. of deuteriomethanol and 42 mg. of 5α-androstane-3,11-dione (VII) was then added. The homogeneous solution was heated under reflux for 10 min. and more deuterium oxide was added until the hot solution became cloudy, whereupon needles (37 mg.) (m.p. 120–120.5°) of **2,2,4,4,9,12-d_6-5α-androstane-3,11-dione (XXVIII)** separated on cooling. Mass spectrometry showed that, in addition to 63% of the d_6-analog XXVIII, there were present the d_4 (3%), d_5 (22%) and d_7 (12%) derivatives. This material (21 mg.) was transformed in the usual manner into the **3-ethylene thioketal XXIX** (28 mg., m.p. 146–146.5°, 6% d_5, 79% d_6, 15% d_7), the difference in deuterium content between the thioketal and the precursor ketone being rationalized on the same basis as observed previously with the thioketal XXIII.

Desulfurization of 26 mg. of the thioketal XXIX with freshly prepared W-7 Raney nickel (from 0.8 g. of alloy) in ethanol (2-hr. reflux) afforded 19 mg. of product, which was shown to be free of olefin by T.L.C. It was heated under reflux for 14 hr. with 100 mg. of sodium hydroxide and 4 cc. of 70% aqueous methanol to back-exchange deuterium from C-9 and C-12. Isolation with ether gave 17 mg. of oil, which crystallized from aqueous methanol to afford 9 mg. of **2,2,4,4-d_4-5α-androstan-11-one (XXX)**, m.p. 48–49°, of 73% isotopic purity, the remainder consisting of 5% d_2-, 13% d_3- and 9% d_5-species.

Δ^4-Androstene-3,11-dione (XXXII).—Bromine (0.21 cc.) in glacial acetic acid (4 cc.) was added dropwise to a solution of 5α-androstane-3,11-dione (VII) (500 mg.) in acetic acid (20 cc.) containing 0.9 cc. of 3 *N* hydrogen bromide in acetic acid. After keeping at room temperature for 25 min., water (30 cc.) was added and the precipitated solid collected. Washing with 50% aqueous methanol and drying *in vacuo* yielded 622 mg. of crude **2α,4α-dibromo-5α-androstane-3,11-dione (XXXI)**, m.p. 92–107°, which could not be purified readily by recrystallization and which was used, therefore, directly in the dehydrobromination step.[30]

Bromine (0.11 cc.) was added to 6 cc. of acetone. When the solution became colorless, it was kept over sodium carbonate (350 mg.) for 20 min. with occasional swirling. The resulting mixture was filtered and the filtrate added to hot acetone (12 cc.) containing 3.2 g. of sodium iodide. The dark brown solution was heated under reflux for 15 min. and 600 mg. of the crude 2,4-dibromide XXXI was added. After heating for 2 hr. under reflux, oxalic acid (650 mg.) was added and heating was continued for a further 50 min. The mixture was diluted with 25 cc. of ethyl acetate, filtered, and the filtrate washed successively with 10% sodium thiosulfate solution, 10% sodium bicarbonate solution and water, dried, and evaporated.

The resulting pale yellow oil (419 mg.) was heated under reflux for 1 hr. with methanol (10 cc.), sodium bisulfite (1.5 g.) and water (5 cc.), and then diluted with water. Extraction with ether provided 202 mg. of a gum, which crystallized on standing. Purification was effected by chromatography on 50 g. of alumina (neutral, activity III) and elution with benzene, containing 30% of hexane, whereupon 127 mg. of crystalline Δ^4-3,11-diketone XXXII was obtained. Recrystallization from hexane led to the analytical specimen (85 mg.) which exhibited m.p. 121–122°, λ_{max} 238 mμ (ε 14,200); $\lambda_{max}^{CHCl_3}$ 5.86, 6.00 and 6.20 μ.

Anal. Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15; mol. wt., 286.4. Found: C, 79.42; H, 9.15; mol. wt., 286 (mass spec.).

5α-d_1- (XXXIVa) and 5β-d_1- (XXXIVb) Androstan-11-ones.—Δ^4-Androstene-3,11-dione (XXXII) (27 mg.) and 10% palladized charcoal catalyst (10 mg.) in cyclohexane (1 cc.) were stirred for 2 hr. at room temperature and atmospheric pressure under an atmosphere of deuterium. Filtration of the catalyst and removal of the solvent left 25.4 mg. of colorless oil, which was heated under reflux for 20 min. with 100 mg. of sodium hydroxide in 4 cc. of 75% aqueous methanol. The resulting C-5 epimeric mixture (20 mg.) of **5-d_1-androstane-3,11-dione (XXXIII)** was treated for 4 min. with 0.02 cc. each of ethanedithiol and boron trifluoride etherate and the crude 3-monothioketal (26.8 mg.) was immediately desulfurized with W-7 Raney nickel catalyst (from 0.6 g. of alloy). Crystallization of the oily product (20 mg.) from methanol afforded 3.0 mg. of crystals, m.p. 106–108° (with sintering from 93°), which on further recrystallization provided 1.5 mg. of pure **5β-d_1-androstan-11-one (XXXIVb)**, m.p. 116–117°, undepressed upon admixture with an authentic sample of 5β-androstan-11-one. Mass spectrometry demonstrated 74% isotopic purity, the remainder representing 21% d_0- and 5% d_2-species.

Investigation of the mother liquor (13 mg.) by gas-phase chromatography on a 5% SE-30 column showed it to contain 5α-d_1- (XXXIVa) and 5β-d_1- (XXXIVb) androstan-11-ones in a ratio of 5:4. Separation was effected at 205° on a 20% SE-30 column, the 5β-isomer XXXIVb having a retention time of 50–53 minutes, and the 5α-isomer XXXIVa having one of 59–61 minutes. The mass spectrum of the latter is reproduced in Fig. 6 and shows the presence of 22% d_0-, 69% d_1- and 9% d_2-species.

In order to determine the ratio of *trans* and *cis* isomers in the catalytic hydrogenation of Δ^4-androstene-3,11-dione (XXXII), a 19-mg. sample of XXXII was hydrogenated in cyclohexane solution (1.5 cc.) with 5 mg. of 10% palladized charcoal and the total product transformed into the 3-monothioketal and directly desulfurized. Gas phase chromatography of the resulting C-5 isomer mixture (12 mg.) of androstan-11-one showed that the ratio of 5β- to 5α-isomer was 1.2:1.

6,6-d_2-5α (XXXVIIa) and 6,6-d_2-5β (XXXVIIb) Androstan-11-ones.—A mixture of Δ^4-androstene-3,11-dione (XXXII) (78 mg.), deuteriomethanol (3 cc.), deuterium oxide (1 cc.) and sodium (150 mg.) was heated under reflux for 30 min., diluted with ether and the organic phase washed with water, dried and evaporated. The brownish gum (72 mg.) was crystallized from hexane to provide 45 mg. of polydeuterated Δ^4-androstene-3,11-dione, m.p. 115–117°, containing 10% d_6-, 54% d_7- and 36% d_8- (XXXV) species.

A portion (38 mg.) of this material was hydrogenated (1 hr.) in cyclohexane solution with 10% palladized charcoal (10 mg.) and the isolated product (33 mg.) back-exchanged by heating under reflux for 7 hr. with 300 mg. of sodium hydroxide and 24 cc. of 33% aqueous methanol. The C-5 epimeric mixture of **6,6-d_2-androstane-3,11-dione (XXXVI)** consisted of a pale yellow gum (33 mg.), the mass spectrum of which showed the presence of 4% d_1-, 81% d_2- and 15% d_3-components.

The entire diketone sample XXXVI was transformed into the 3-thioketal (41 mg.) and desulfurized to afford 31 mg. of a partially crystalline product, which by gas-phase chromatography was shown to consist of a 2.5:1 ratio of 5β (XXXVIIb) to 5α (XXXVIIb) isomer. Recrystallization from acetone–methanol yielded 7.1 mg. of the 5β-11-ketone XXXVIIb, m.p. 112–116°, raised to 118–120.5° (4 mg.; 4% d_1, 76% d_2, 20% d_3) upon one recrystallization.

A portion of the mother liquor was resolved by gas-phase chromatography to provide the stereochemically pure **6,6-d_2-5α-androstan-11-one (XXXVIIa)**, the mass spectrum of which indicated the presence of 4% d_1, 71% d_2- and 25% d_3-species.

9α-Bromo-5α-androstan-11-one (XXXVIII).—A solution of 50% hydrogen bromide in acetic acid (4 drops) was added to a solution of 5α-androstan-11-one (IX) (90 mg.) in acetic acid (0.6 cc.), followed by the dropwise addition of 0.02 cc. of bromine in acetic acid (0.5 cc.). After keeping the solution for 1 hr. at room temperature in the dark in a current of nitrogen, the crude product (115 mg.), isolated in the usual manner by dilution with water and ether extraction, was chromatographed on 50 g. of neutral alumina (activity II), the column being developed with petroleum ether (b.p. 30–60°). After an initial 80 cc., the next 20 cc. of eluent produced 49 mg. of crystals, suitable for the dehydrobromination step. Recrystallization from methanol–ether yielded 25 mg. of 9α-bromo-5α-androstan-11-one (XXXVIII), m.p. 71–72°; R.D. in methanol (*c* 0.12): $[\alpha]_{589}$ +236°, $[\alpha]_{350}$ +3240°, $[\alpha]_{295}$ −3640°, $[\alpha]_{250}$ −2540°.

Anal. Calcd. for $C_{19}H_{29}BrO$: C, 64.59; H, 8.27; Br, 22.60. Found: C, 64.49; H, 8.30; Br, 22.37.

Δ^8-5α-Androsten-11-one (XXXIX).—Calcium carbonate (100 mg.) was added to a solution of crude 9α-bromo-5α-androstan-11-one (XXXVIII) (125 mg.) in dimethylacetamide (2 cc.) and the resulting suspension was heated under reflux for 15 min. After isolation in the usual manner, the product (96 mg.) in 5 cc. of petroleum ether (b.p. 60–80°) was adsorbed onto a column of neutral alumina (25 g., activity II) and the column developed with 25 cc. of petroleum ether. Further development with the same solvent (25 cc.) afforded 7 mg. of crude, crystalline **12α-bromo-5α-androstan-11-one (XLIII)** (R.D. extrema in methanol: $[\alpha]_{344}$ −1836°, $[\alpha]_{296}$ +2363°), while the next 25 cc. removed 21 mg. of oil. Finally, a crystalline product (39 mg.) was eluted by petroleum ether (150 cc.) and recrystallized from aqueous methanol to provide 21 mg. of **Δ^8-5α-androsten-11-one (XXXIX)** as large needles, m.p. 113–114°, $[\alpha]_D$ +180° (*c* 1.3) $\lambda_{max}^{CHCl_3}$ 6.07 and 6.24 μ, λ_{max} 253 mμ (ε 8,300).

Anal. Calcd. for $C_{19}H_{28}O$: C, 83.77; H, 10.36. Found: C, 83.74; H, 10.36.

8β-d_1-5α-Androstan-11-one (XLI).—Δ^8-5α-Androsten-11-one (XXXIX, 19 mg.), dissolved in a 1:1 mixture (0.6 cc.) of dioxane–ether, was added dropwise to a solution of lithium metal (5 mg.) in deuterioammonia (1 cc.),[55] while cooling in an acetone–

(55) Deuterioammonia was generated (see ref. 37) by adding 7 cc. of deuterium oxide over a period of 8 min. to 12 g. of magnesium nitride and leading the liberated deuterioammonia through a trap, maintained at room temperature, directly to the reaction vessel, which was cooled in an acetone–Dry Ice bath. The intermediate trap served to condense most of the deuterium oxide vapor which is generated by the vigorously exothermic reaction. The deuterioammonia so obtained (*ca.* 1 cc.) certainly contains traces

Dry Ice bath. Dry ether (2 cc.) was then added and, after stirring for 5 min., the blue color was destroyed by the addition of deuteriomethanol (0.5 cc.). Dilution with ether, washing with water, drying and evaporation afforded 19.6 mg. of a colorless gum, which was homogeneous by gas-phase chromatography and free of 5α-androstan-11-one. This material, most likely 5α-androstan-11α-ol, was oxidized in acetone solution (0.5 cc.) with 5 mg. of chromium trioxide in 20% sulfuric acid (0.02 cc.) to give an oil (18 mg.) which was chromatographed on 2 g. of neutral alumina (activity II). Elution with petroleum ether provided 9.6 mg. of crystalline **8β,9α-d_2-5α-androstan-11-one (XL)**, which exhibited m.p. 48–49.5° (4.1 mg.; 27% d_1, 63% d_2, 10% d_3) after one recrystallization from aqueous methanol at −15°.

A solution of 3.8 mg. of the 8β,9α-d_2-ketone XL and 80 mg. of sodium hydroxide in 4 cc. of 50% aqueous methanol was heated under reflux for 4 days and the product crystallized from aqueous methanol (−15°) to provide 2.1 mg. of **8β-d_1-5α-androstan-11-one (XLI)**, m.p. 49–49.5°. The mass spectrum (Fig. 5) demonstrated 93% isotopic purity, the contaminants being represented by 5% d_0- and 2% d_2-analogs.

9α-d_1-5α-Androstan-11-one (XLII). (a) **By Zinc–Deuterioacetic Acid Debromination of 9α-Bromo-5α-androstan-11-one (XXXVIII).**—9α-Bromo-5α-androstan-11-one (XXXVIII, 13 mg., m.p. 71–72°), dissolved in 2 cc. of ether and 0.5 cc. of deuterioacetic acid, was stirred at room temperature with 35 mg. of zinc dust for 4 hr., at which time T.L.C. (developed with 1:1 benzene–petroleum ether) indicated that reduction was complete and 5α-androstan-11-one was the sole product. The suspension was filtered and the residue washed well with ether. The filtrate was washed with sodium bicarbonate solution and water, dried and evaporated to give a colorless oil (11.8 mg.), which crystallized on standing. The 9α-d_1-5α-androstan-11-one (XLII) so obtained had m.p. 48–49° and its mass spectrum showed the isotopic composition 38% d_0 and 62% d_1. In Fig. 4, the contribution of the non-labeled species has been subtracted.

(b) **By Deuterium Bromide-Catalyzed Enolization of 5α-Androstan-11-one (IX).**—5α-Androstan-11-one (IX, 35 mg.) was added to a solution of hydrogen bromide (20 mg.) in deuterioacetic acid (5 cc.) and the homogeneous solution was allowed to stand at room temperature, small samples being removed for mass spectrometric assay after 15 min. (97% d_0, 3% d_1); 4 hr. (83% d_0, 16% d_1, 1% d_2) and 16 hr. (52% d_0, 45% d_1, 3% d_2). These aliquots were worked up by pouring into ether and washing twice each with 10% sodium bicarbonate solution and water, drying and evaporating, the residual colorless crystals being used directly for mass spectrometry.

The 16-hr. sample (30 mg.) in deuterioacetic acid (4.9 cc.), containing 49 mg. of hydrogen bromide, was kept at room temperature for a further 28 hr. Isolation of a 20-mg. sample after this time gave 9α-d_1-5α-androstan-11-one (XLII), m.p. 50–51° (16% d_0, 76% d_1, 8% d_2), while the remaining product was isolated after 150 hr. (11% d_0, 61% d_1, 25% d_2, 3% d_3).

12α-d_1-5α-Androstan-11-one (XLIV).—To a solution of 5α-androstan-11-one (IX) (66 mg.) in 0.5 cc. of acetic acid was added successively 0.25 cc. of 35% hydrogen bromide in acetic acid solution and bromine (0.014 cc.) in acetic acid (1.0 cc.). After keeping at room temperature for 2.5 days, the product was extracted with ether and washed with water, sodium bicarbonate solution and again water, dried and evaporated. The pale brown residue (986 mg.) crystallized on standing and upon recrystallization from methanol afforded **12α-bromo-5α-androstan-11-one (XLIII)** as colorless leaflets (43 mg.), m.p. 107–107.5°; R.D. in methanol (c 0.10): $[\alpha]_{589}$ −59°, $[\alpha]_{344}$ −2970°, $[\alpha]_{295}$ +3860°, $[\alpha]_{270}$ +3160°.

Anal. Calcd. for $C_{19}H_{29}BrO$: C, 64.59; H, 8.27; Br, 22.60. Found: C, 64.67; H, 8.34; Br, 22.22.

The 12α-bromo ketone XLIII (7.7 mg.) was stirred at room temperature for 4 hr. with 20 mg. of zinc dust, 1 cc. of ether and 0.25 cc. of deuterioacetic acid. After working up in the above-described manner, there was isolated 5 mg. of **12α-d_1-5α-androstan-11-one (XLIV)** m.p. 49–50° (47% d_0, 53% d_1), the contribution of the non-deuterated contaminant having been subtracted in plotting the mass spectrum reproduced in Fig. 2.

of deuterium oxide, which is probably responsible for the reduction of the α,β-unsaturated ketone directly to the saturated alcohol.

9α,12α-d_2-5α-Androstan-11-one (XLV).—5α-Androstan-11-one (IX) (29 mg.) was heated under reflux for 20 min. with sodium (60 mg.) dissolved in deuteriomethanol (2 cc.) and deuterium oxide (0.5 cc.). Sufficient deuteriomethanol was then added to maintain homogeneity at room temperature. Upon cooling to −15°, the 9α,12α-d_2-11-ketone XLV (22 mg.) separated as needles, m.p. 51.5–52.5°, of 91% isotopic purity, the contaminants being 2% d_1- and 7% d_3-species.

9α,12,12-d_3-5α-Androstan-11-one (XLVI).—The equilibration of 5α-androstan-11-one (IX) was performed as in the preceding paragraph except that the reflux time was extended to 4 days, whereupon mass spectrometric analysis showed that the crystals (m.p. 50–51°) consisted of 9% d_2- and 91% d_3-(XLVI) species.

12β-d_1-5α-Androstan-11-one (XLVII).—9α,12,12-d_3-5α-Androstan-11-one (XLVI, 18 mg.) and 200 mg. of sodium hydroxide in 7 cc. of 70% aqueous methanol were heated under reflux for 65 min. The crystalline residue (17 mg.), obtained by ether extraction, was recrystallized from aqueous methanol to furnish 9 mg. of the 12β-d_1-11-ketone XLVII, m.p. 51–52°, the mass spectrum (Fig. 3) of which showed the presence of 11% d_0 and 4% d_2 isotopic contaminants.

Ring-C Labeled Δ²-5α-Androsten-11-ones (Table III).—Equilibration of 6.4 mg. of Δ²-5α-androsten-11-one (X) in the above-described manner for 20 min. led by direct crystallization to 3.7 mg. of the **9α,12α-d_2-analog XLVIII**, m.p. 88–89°, containing 2% d_1- and 10% d_3-species as contaminants. When the reflux time was extended to 4 days, the **9α,12,12-d_3-11-ketone XLIX** (m.p. 90–90.5°) proved to be of 72% isotopic purity, accompanied by 3% d_1- and 25% d_2-species. Back-exchange of this material with sodium hydroxide in aqueous methanol (57-min. reflux) provided **12β-d_1-Δ²-5α-androsten-11-one (L)** (m.p. 89–90°) of 76% d_1-content, mixed with 19% d_0- and 5% d_2-species.

9α,12α-d_2-5α-Pregnan-11-one (LII).—A solution of 3.0 mg. of 5α-pregnan-11-one (LI)[16] in 2 cc. of deuteriomethanol and 0.2 cc. of deuterium oxide containing 20 mg. of sodium was heated under reflux for 30 min. On addition of a few drops of deuterium oxide and slow cooling, there was obtained 2.0 mg. of 9α,12α-d_2-5α-pregnan-11-one (LII), m.p. 111–113.5°, the mass spectrum (Fig. 9) of which showed the presence of 6% d_1-, 86% d_2- and 8% d_3-species.

17,17-d_2-5α-Androstan-11-one (LV).—A mixture of 70 mg. of 5α-androstane-11,17-dione-3β-ol acetate 17-ethylene thioketal (IV) and freshly prepared deuterium-containing Raney nickel (from 1 g. of alloy) in 4 cc. of deuteriomethanol was heated under reflux for 4 hr. The isolated product (48 mg.) and potassium hydroxide (200 mg.) in 70% aqueous methanol were heated under reflux for 17 hr. and the usual work-up gave crude **17,17-d_2-5α-androstan-3β-ol-11-one (LII)** as a crystalline solid of the isotopic composition: 2% d_0, 12% d_1, 40% d_2, 37% d_3, 9% d_4.

Tosyl chloride (80 mg.) was added to a solution of 42 mg. of the above hydroxyketone LIII in 0.2 cc. of pyridine. The solution was kept overnight at room temperature and the tosylate LIV (80 mg.) isolated in the usual manner by extraction with ether. A sample (47 mg.) of the crude tosylate was reduced (20 hr., room temperature) with lithium aluminum hydride (60 mg.) in ether (3 cc.) and the resulting crude 17,17-d_2-5α-androstan-11β-ol (21 mg.)[56] was oxidized in acetone solution (0.3 cc.) with chromium trioxide (5 mg.) in 20% sulfuric acid (0.02 cc.). Traces of polar impurities in the oxidation product were removed by filtration (in 9:1 hexane–benzene solution) through alumina; crystallization from aqueous methanol gave the desired **17,17-d_2-5α-androstan-11-one (LV**, 40% isotopic purity), m.p. 45–47°, which contained 3% d_0, 12% d_1, 35% d_3 and 9% d_4 isotopic contaminants.

17,17-d_2-Δ²-5α-Androsten-11-one (LVI).—A benzene solution of 30 mg. of the above-described crude tosylate LIV was treated with basic alumina as outlined in the unlabeled series (VI → X) to give, after recrystallization from aqueous methanol, 17,17-d_2-Δ²-5α-androsten-11-one (LVI), m.p. 89–91°, mass spectrometry showing the presence of 1% d_0-, 12% d_1-, 40% d_2- (LVI), 36% d_3- and 11% d_4-species.

(56) Pure 5α-androstan-11β-ol was prepared in a similar manner in the unlabeled series (from VI) and crystallized as shining plates (m.p. 91–92°) from aqueous methanol. *Anal.* Calcd. for $C_{19}H_{32}O$: C, 82.54; H, 11.66. Found: C, 82.57; H, 11.90.